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RESEARCH PAPERS

Characterization of the adjuvant activity of lipid extracts of *Sarcopeltis skottsbergii* and *Iridea cordata* associated with recombinant protein rCP01850 as an immunogen against caseous lymphadenitis

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Highlights

- The macroalgae lipid extracts presented fatty acids with promising adjuvant activity in recombinant vaccines
- The macroalgae lipid extracts induced increased total IgG production
- The macroalgae lipid extracts induced increased IL-10 and interferon-γ production

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KEYWORDS
Natural products; Recombinant vaccines; *Corynebacterium pseudotuberculosis*; Fatty acids; Seaweed.

Abstract: Fatty acids from macroalgae display various promising biological activities, including immunomodulatory activity. Here, we characterized the adjuvant potential of macroalgae lipid extracts in a recombinant vaccine against caseous lymphadenitis. Female BALB/c mice were divided into seven groups and immunized twice (Days 0 and 21) with 0.9% saline solution (G1); rCP01850 + saponin (G2); rCP01850 + *Sarcopeltis skottsbergii* gametophyte phase (SFG) (G3); rCP01850 + *Sarcopeltis skottsbergii* cystocarp phase (SFC) (G4); rCP01850 + *Iridaea cordata* cystocarp phase (IFC) (G5); rCP01850 + *Iridaea cordata* tetrasporophyte phase (IFT) (G6); or rCP01850 alone (G7). Blood samples were collected for total IgG, IgG1, and IgG2a quantification. Moreover, another assay measured the IFN-γ and IL-10 levels produced by each experimental group through *in vitro* stimulation of splenocytes with rCP01850. Groups G5 and G6 showed the highest antibody levels after day 42. Groups G3, G4, G5, and G6 significantly increased IgG1 levels when compared to G1 and G7, with no statistical difference between G3, G4, G5, and G6. Meanwhile, G5 and G6 showed a significantly higher increase in IgG2a levels than the other groups on day 42. Groups G3, G4, G5, and G6 significantly produced IL-10, while G3 and G5 showed the highest levels of IFN-γ ($p < 0.05$). We concluded that the lipid extracts of *Sarcopeltis skottsbergii* and *Iridaea cordata*, when associated with rCP01850, elicited both humoral and cellular immune responses and are, therefore, promising candidates as adjuvants in vaccines against caseous lymphadenitis.

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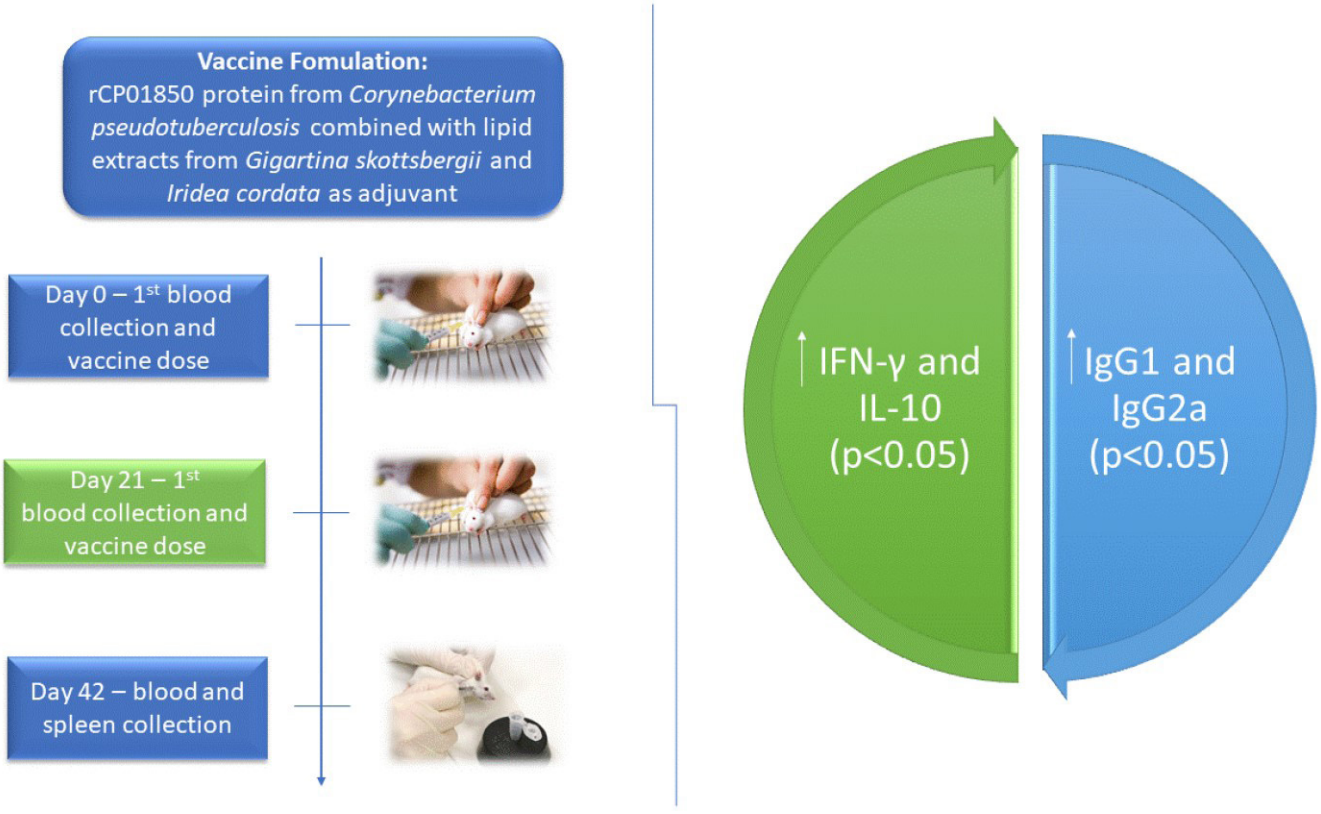
Graphical Abstract



Introduction

The role of adjuvants can be defined in two ways: (i) substances that act by delivering the antigen to the lymph nodes, assisting in its absorption and exposure to antigen-presenting cells (APCs), and (ii) stimulate the recruitment and activation of APCs and T cells (Charerntantanakul, 2020). In addition, they are also capable of providing an enhanced immune response, consequently lowering the number of vaccine doses used for immunization and reducing the production costs of vaccines (Tregoning et al., 2018).

In this context, developing new vaccine formulations using different types of adjuvants stands out as a strategy to protect against several infectious diseases, such as caseous lymphadenitis (CLA), a chronic disease that affects numerous animal species, especially small ruminants such as goats and sheep (Silva et al., 2020). The severity of this disease can be seen in its negative economic impacts on small ruminant farming due to its effect on meat, wool, and milk production and because it leads to impaired reproductive performance and carcass condemnation. Although commercial vaccines are available, they fail to provide full protection, reinforcing the need to develop more effective vaccine formulations (Sobrinho Santos et al., 2018). Therefore, we highlight the importance of finding more efficient adjuvants that strengthen and improve immune response in the fight against CLA (Nazarizadeh et al., 2022).

Subantarctic macroalgae are valuable sources of biomolecules, which are of interest to research aimed at developing new vaccine formulations (Chiboub et al., 2019). These organisms can adapt to inhospitable environments, such as the subantarctic region, and as a result, synthesize diverse metabolites that are considered biologically active (Saadaoui et al., 2020). Interestingly, the same macroalgae species can have diverse biomolecule profiles depending on factors such as climate change and their development/life stage, and this could implicate changes in their biological activities. Therefore, it is essential to characterize the possible applications of macroalgae, not only for a certain species but also for their different developmental phases (Barbosa et al., 2023).

Studies aiming at possible applications of subantarctic macroalgae in the biomedical industry have been performed, including one for their immunomodulatory activity (Jin et al., 2018). Barbosa et al. (2020), showed that green algae have an immunostimulatory role by producing pro-inflammatory cytokines, such as TNF-α and IL-6. These results corroborate how new adjuvants could be obtained from these marine organisms. Thus, red subantarctic macroalgae, such as *Sarcopeltis skottsbergii* and *Iridaea cortada*, whose bioactive components have demonstrated biological activities, are promising candidates for producing new adjuvants.

S. skottsbergii, previously known as *Gigartina skottsbergii*, has already shown interesting pharmacological potential. Its antiparasitic activity was demonstrated in the study conducted by Barbosa et al. (2023), in which different *S. skottsbergii* development forms reduced the viability of *Trichomonas vaginalis* trophozoites by up to 100%. Moreover,

Martins et al. (2018) demonstrated that *I. cordata* had significant antitumoral activity, inhibiting 95.6% of human carcinoma cell growth and beneficially stimulating the growth of normal cells. Therefore, using metabolites from marine macroalgae as adjuvants in vaccine formulations is a promising strategy.

In addition to selecting a beneficial adjuvant, choosing the vaccine target is essential for efficiency (Coolen et al., 2019). In this sense, associating macroalgae extracts with the recombinant acid phosphatase rCP01850 from *Corynebacterium pseudotuberculosis*, the causative agent of CLA, becomes a viable strategy. Even more, considering rCP01850 has already been classified as one of the most promising targets for developing vaccines against CLA (Rezende et al., 2020). In a study conducted by (Bezerra et al., 2020), rCP01850 adjuvanted with the Brazilian red propolis hydroalcoholic extract led to significant protection levels (70%) against CLA in mice.

Therefore, this study focused on understanding whether extracts from subantarctic macroalgae, specifically *Sarcopeltis skottsbergii* and *Iridaea cordata* at various stages of development, have adjuvant activity when associated with rCP01850 in recombinant vaccines for CLA.

Materials and methods

Collection of subantarctic macroalgae

Iridaea cordata specimens in the tetrasporophyte and cystocarp stages and *Sarcopeltis skottsbergii* specimens in the gametophyte, tetrasporophyte, and cystocarp stages were collected in the Punta Arenas region of Chile (Latitude: – 53,1667, Longitude: – 70,9333 53° 10′ 0″ South, 70° 55′ 60″ West). After that, specimens were washed (distilled H_2O) and morphologically identified. Subsequently, the samples were lyophilized, sprayed, packed in dark plastic bags, and placed in a desiccator to protect them from heat, light, and moisture before analysis.

Producing the lipid extracts

Fatty acids (FAs) were obtained from the macroalgae samples as described by (Bligh & Dyer, 1959). Briefly, 1 g of algae biomass was added to a solution consisting of methanol (20 mL), chloroform (10 mL), and a 1.5% (w/v) sodium sulfate aqueous solution (10 mL) and then stirred at room temperature for 30 min. Subsequently, the samples were transferred to conical tubes and centrifuged at 3000 rpm for 30 min. Then, the lower layer of the organic phase was recovered and dried under reduced pressure. The procedure was performed in triplicate (n = 3).

The FAs derivatization process was performed as described by Moss et al. (1974). The extracted material was refluxed with a 0.5 M sodium hydroxide methanol solution at 100 °C for 5 min. Then, 5 mL of a 14% boron trifluoride methanol solution was added to the sample and refluxed at 100 °C for 5 min. Subsequently, the system was cooled down with 3 mL of a sodium chloride-saturated aqueous solution, and 20 mL of n-hexane was added to the solution. After that, the solution was transferred to a separatory funnel to isolate the upper organic phase, which was filtered over anhydrous sodium sulfate and dried under reduced pressure. The procedure was performed in triplicate (n = 3).

Instrumentation and fatty acids quantification

After extracting and derivatizing the FAs, the samples were diluted in n-hexane and introduced into a GC-FID model GC-2010 (Shimadzu, Kyoto, Japan), using nitrogen as carrier gas and an SP-2560 capillary column (100 m x 0.25 mm x 0.2 µm) from Supelco (Bellefonte, USA). The temperature was set at 140 °C, with an increasing rate of 4 °C per minute up to 240 °C, which was maintained for 10 min. Thus, the total run was 40 min. The injector was maintained at 260 °C, while injections were performed in split mode (1:100). FAs were identified and quantified through comparison to a FAME 37-Mix standard using GC Solution software (Shimadzu, Kyoto, Japan).

Chemicals and standards

Methanol, chloroform, sodium chloride, anhydrous sodium sulfate, and sodium hydroxide were purchased from Labsynth (Diadema, Brazil), while HPLC grade n-hexane was purchased from JT Baker (Phillipsburg, USA). Boron trifluoride and nonadecanoic methyl ester (C19:0) methanolic solutions were purchased from Sigma-Aldrich (St. Louis, USA). The fatty acid methyl esters standard blend 37 was obtained from Supelco (Bellefonte, USA). All standards and chemicals were of analytical grade.

Experimental animals and ethical aspects

Female BALB/c mice (6-8 weeks) were provided by the Central Animal Facility of the Federal University of Pelotas and used in the experiments. The mice remained accommodated in cages with water and food *ad libitum* in a light/dark cycle (12/12h) at a room temperature of approximately 21 °C. The entire experimental procedure was conducted following the guidelines of the Brazilian College of Animal Experimentation (COBEA). The project was submitted to the Animal Experimentation Ethics Committee (CEEA) of the Federal University of Pelotas and approved under number 12522/2019.

rCP01850 heterologous expression

The vaccine target, a protein from *C. pseudotuberculosis* (rCP01850), was expressed following the methodology previously established by our group (Rezende et al., 2016) and used in association with subantarctic macroalgae extracts as adjuvants. Recombinant protein expression occurred in *Escherichia coli* BL21 through induction with 1 mM of IPTG. The culture remained under agitation for 3 h at 37 ºC in an orbital shaker. Then, a Western blot (WB) was performed using the anti-6×His tag monoclonal antibody conjugated with peroxidase (HRP) (Sigma–Aldrich, USA) to confirm recombinant protein expression. WB was followed by purification through nickel affinity chromatography on a

sepharose column (HisTrap, GE Healthcare). Protein purity was determined using a 12% SDS-PAGE gel, and its concentration was determined using the BCA kit (Pierce).

Vaccine formulations

The immunization assay was performed according to Bezerra et al. (2021), with some modifications. For that, 35 mice were allocated to 7 groups of 5 animals each. Control groups were inoculated with saline (0.9% NaCl, 200 µL) (G1), saponin (7.5 µg) combined with 50 µg rCP01850 diluted in saline (0.9% NaCl, 200 µL) (G2), and 50 µg rCP01850 diluted in saline (0.9% NaCl, 200 µL) (G7). Experimental groups were inoculated with with vaccine formulations (200 µL, total volume) containing rCP01850 (50 µg recombinant protein per dose), and lipid extracts (10 µg) from *Sarcopeltis skottsbergii* gametophyte phase (SFG) (G3); *Sarcopeltis skottsbergii* cystocarp phase (SFC) (G4); *Iridaea cordata* cystocarp phase (IFC) (G5); *Iridaea cordata* tetrasporophyte phase (IFT) (G6), respectively, diluted in sterile saline (0.9% NaCl). All groups were immunized subcutaneously. Blood samples were collected on days 0, 21, and 42 after immunization via the submandibular route. After clotting, the blood was centrifuged at 1,500 g for 15 min, and the serum was obtained and stored at -20 °C until antibody level assays were performed.

Characterizing the humoral immune response

Serum samples from immunized mice were submitted to indirect ELISA, in which total IgG, IgG1, and IgG2a antibodies were quantified through their interaction with the target protein rCP01850. 96-well polystyrene plates (Maxisorp-Nunc) were coated with carbonate-bicarbonate buffer (100 µL/well) (pH 9.8) containing the rCP01850 protein (100 ng/well). After that, the plates were incubated for 18 h at 4 °C and washed three times with PBS-T (1X PBS, pH 7.4, 0.1% Tween 20). The plates were incubated with 5% skimmed milk in PBS (200 µL/well) for 2 h at 37 °C to block unbonded portions of the wells and avoid unspecific binding. Then, the plates were washed three more times with PBS-T. All mice serum samples were diluted in PBS (v/v, 1:50) and added to the plates (100 µL/well) in duplicate.

Plates were incubated for 1 h at 37 °C. After three washes with PBS-T, 100 µL/well of peroxidase-conjugated anti-mouse IgG Total (Sigma-Aldrich) (1:5000 in PBS-T) was added for total IgG detection. For IgG1 and IgG2a detection, 100 µL/well of goat anti-mouse IgG1 (1:5000 in PBS-T) or goat anti-mouse IgG2a (1:2000 in PBS-T) was added. The plates were incubated at 37 °C for 1 h and washed three times with PBS-T. After that, 100 µL/well of peroxidase-conjugated anti-goat IgG (Sigma-Aldrich) (1:5000 in PBS-T) was added. The plates were incubated for 1 h at 37 °C and washed five times with PBS-T.

The reaction was developed by adding 100 µL/well of a substrate-chromogen solution [o-phenylenediamine dihydrochloride; OPD tablets (Sigma-Aldrich) in 0.4 mg/mL phosphate-citrate buffer containing 0.04% of 30% hydrogen peroxide, pH 5.0] and incubating the plates at room temperature in the dark for 15 min. The reaction was stopped by adding an H_2SO_4 solution (50 µL/well). Optical density (OD) was determined using a microtiter plate reader (Microplate Reader Mindray MR – 96A) set at 492 nm.

Characterizing the cellular immune response

For this assay, female BALB/c mice (6-8 weeks) were allocated to seven experimental groups (6 mice per group). Forty-two days after the first vaccine dose, the animals were euthanized, and their spleens were aseptically removed and homogenized for splenocyte isolation. Cells were grown in Dulbecco MEM (DMEM) high glucose medium supplemented with 10% fetal bovine serum. After counting in a Neubauer chamber, the cell concentration was adjusted to 5×10^6 cells/mL, and viability was determined by exclusion with trypan blue dye (Vetec, BRL). Then, 3 mL of cells were seeded into 12-well plates (MaxiSorp, Nunc, USA) in triplicate.

Then, cultured cells were stimulated for cytokine production through the addition of the protein rCP01850 from *C. pseudotuberculosis* (10 µg/mL), concanavalin A (10 µg/mL) as a positive control, or culture medium as a negative control. The cells were then incubated in a CO_2 (5%) incubator at 37 °C for 48 h. Subsequently, the cultures were centrifuged, and the supernatants were collected and stored at – 20 °C for cytokine quantification. A sandwich ELISA was used to quantify murine IFN-γ and IL-10 (BD OpTEIA®, USA), and the assays were performed according to the manufacturer's instructions. Results were expressed in pg/mL.

Statistical analysis

The data were submitted to GraphPad Prism version 5 software for Windows (GraphPad Software) for statistical analysis. For antibody level assays and cytokine quantification assays, differences between groups were analyzed by unidirectional ANOVA followed by Tukey's test for multiple comparisons. Differences were considered statistically significant when $p < 0.05$.

Results

The characterization of the lipid extracts was performed using flame ionization by gas chromatography (GC-FID) (Table 1). The chemical composition profile of the extracts evaluated here showed that IFC consisted of 31.87% saturated fatty acids (SFAs), 16.57% monounsaturated fatty acids (MFAs), and 51.56% polyunsaturated fatty acids (PFAs), while IFT showed 30.08% SFAs, 13.74% MFAs, and 56.18% PFAs. In turn, SFG extract comprised 50.29% SFAs, 15.30% MFAs, and 34.41% PFAs, and GFC consisted of 59.92% SFAs, 18.48% MFAs, and 21.6% PFAs.

The rCP01850 protein was expressed in inclusion bodies in the *E. coli* BL21 strain, and its recovery was performed under denaturation conditions with 8 M urea. After purification, rCP01850 had a yield of 6.2 mg/mL. The protein identity was confirmed by western blotting using the anti-6xHis monoclonal antibody, and the band presented the expected size of approximately 33 kDa (Figure 1).

The humoral immune response was analyzed on days 0, 21, and 42 through an indirect ELISA with sera from the

Table 1. Fatty acid composition of lipid extracts from *Sarcopeltis skottsbergii* and *Iridaea cordata* at different development stages identified by gas chromatography-flame ionization detector (GC-FID).

Fatty acids	*Sarcopeltis skottsbergii*		*Iridaea cordata*	
	Gametophyte*	Cystocarp*	Cystocarp*	Tetrasporaphyte*
Lauric acid (12: 0)	1.07	-	0.65	0.46
Myristic acid (14: 0)	5.52	6.53	2.64	2.55
Myristoleic acid (C14:1)	1.14	-	-	-
Pentadecanoic acid (C15:0)	1.40	-	0.55	-
Palmitic acid (C16:0)	34.46	39.74	23.92	24.71
Palmitoleic acid (C16:1)	2.56	4.45	4.06	2.16
Heptadecanoic acid (C17:0)	1.65	-	0.48	-
Stearic acid (C18: 0)	6.19	13.65	3.10	2.36
Oleic acid (C18:1n9c)	11.62	14.03	10.08	9.48
Linoelaidic acid (C18:2*n*6t)	-	-	2.20	1.19
Linoleic acid (C18:2n6c)	2.15	6.59	4.52	3.71
Linolenic acid (C18:3n3)	-	-	5.21	3.42
Ketoleic acid (C20:1*n*9c)	-	-	0.51	0.65
Eicosadienoic acid (C20:2)	-	-	2.17	2.16
eicosatrienoic cis-8,11,14-acid (C20:3n6)	2.22	-	1.99	1.60
arachidonic acid (C20: 4n6)	15.50	6.86	17.29	21.92
Eicosapentaenoic acid (C20: 5n3)	14.56	8.15	17.65	21.45
Erucic acid (C22:1*n*9c)	-	-	0.77	0.54
Lignoceric acid (C24: 0)	-	-	0.53	-
Nervonic acid (C24:1*n*9)	-	-	1.15	0.91

*Values expressed as percentages.

immunized animals (Figure 2). The results showed that total anti-rCP01850 IgG antibody levels were significantly increased ($p < 0.05$) on day 42 in groups G3, G4, G5, and G6 (all containing a lipid extract as adjuvant) when compared to control. Moreover, as shown in Figure 2B, IgG1 antibody production showed significant ($p < 0.05$) levels on day 42 for all groups using lipid extracts as adjuvants (G3-G6).

When observing IgG2a production (Figure 2C), G3, G4, G5, and G6 showed a significant increase in antibody levels when compared to G1 and G7, with G5 and G6 presenting the highest levels of this isotype when compared to the other groups.

We also measured IFN-γ and IL-10 levels for each experimental group using a sandwich ELISA to understand whether vaccine formulations elicited cellular immune response. IFN-γ levels significantly increased in groups G2, G3, and G5 compared to the negative control ($p < 0.05$), but there was no significant difference between these experimental groups. In turn, G4, G6, and G7 did not induce significant IFN-γ production in comparison to the control (Figure 2). Meanwhile, IL-10 levels were up-regulated in all experimental groups (G2-G7), but G5 and G6 enhanced IL-10 concentration considerably more when compared to the other groups ($p < 0.05$) (Figure 3).

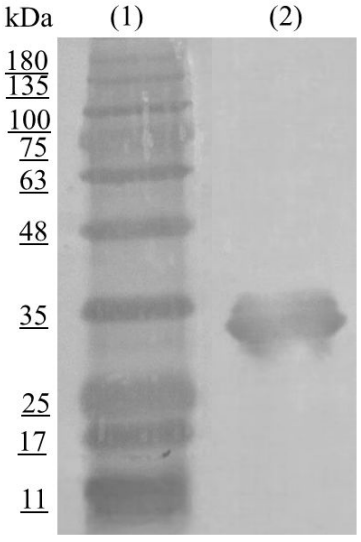


Figure 1. Confirming the identity of *Corynebacterium pseudotuberculosis* recombinant protein CP01850 by Western blotting with anti-6xHis tag monoclonal antibody. 1: Prestained marker (Prestained PageRuler Protein Ladder, Thermo Fisher); 2: rCP01850. The recombinant protein had a reactive band of approximately 33 kDa.

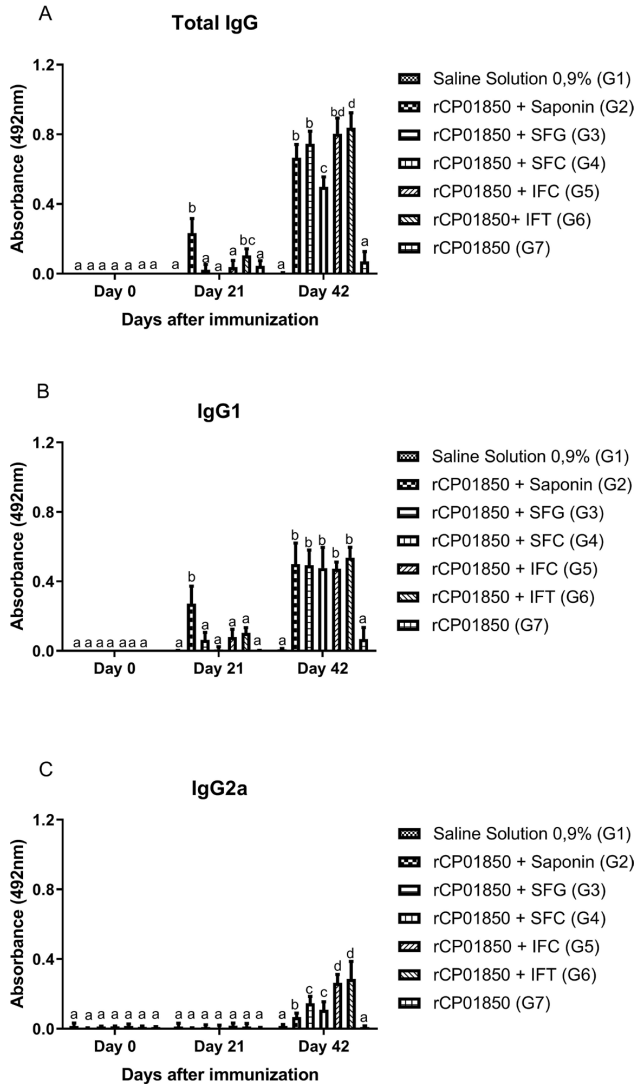


Figure 2. Total IgG (A), IgG1 (B), and IgG2a (C) antibody levels in mice immunized with different vaccine formulations. The results are presented as mean and standard deviation (bars) of the absorbances (492nm) found in the indirect ELISA for each experimental group. Blood was collected on days 0, 21, and 42 after the 1st immunization. Different letters within the same day represent significantly different groups ($p < 0.05$).

The animals were immunized twice within a 21-day interval, and after the 42nd day, splenocytes were obtained, cultured, and stimulated in vitro for cytokine production. Data are presented as mean ± standard deviation. Different letters show a significant difference between groups at $p < 0.05$.

Discussion

In the present study, we characterized the adjuvant potential of lipid extracts obtained from subantarctic macroalgae as a new strategy for developing efficient vaccine formulations in the fight against infectious and contagious diseases, as is the case for caseous lymphadenitis, which we addressed in this work.

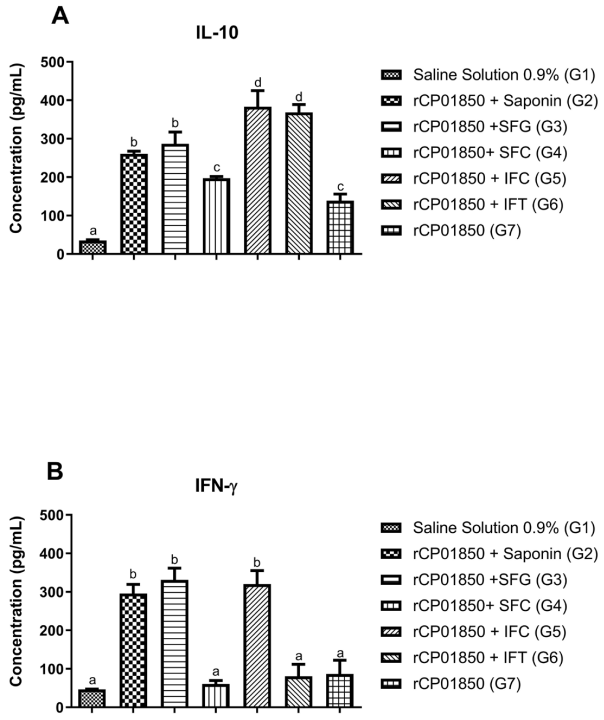


Figure 3. *In vitro* production of interleukin 10 (A) and interferon-gamma (B) by splenocytes obtained from mice immunized with vaccine formulations containing rCP01850 associated with lipid extracts of *Sarcopeltis skottsbergii* and *Iridaea cordata* at different development stages.
Different letters within represent significantly different groups ($p < 0.05$)

Recently, many studies have described how lipid compounds, such as fatty acids, are important for immune activity since immune cells have a high fatty acid content. Moreover, fatty acids are involved in numerous biological activities, including those influencing immune activity (Miles et al., 2021), and are helpful against diseases and infections (Shakoor et al., 2021).

Here, when investigating the composition of four macroalgae extracts (SFG, SFC, IFC, and IFT), we identified several classes of fatty acids in their compositions, many of which have already been shown to have immunomodulatory activity. These findings corroborate fatty acids as potential adjuvants.

Several studies have focused on the immunostimulatory effects of fatty acids, corroborating our study's hypothesis. For instance, Zhou et al. (2021) demonstrated how monounsaturated fatty acids, such as oleic acid and palmitic acid, play a crucial role in B cell metabolism, directly impacting humoral response. Moreover, Yue et al. (2022) indicated that polyunsaturated fatty acids, such as omegas, play a part in boosting humoral immune response and increasing the number of type 1 (Th1) CD4+ T Helper cells. Fatty acids can also partake in immunoregulation when interacting with T cells by modulating their effector functions (Radzikowska et al., 2019). Moreover, mice submitted to a fatty acid-rich diet have shown increased TCD4+ and TCD8+ cell growth in the spleen (Gutiérrez et al., 2019). As previously described, the development phases of macroalgae can present significant differences in their biochemical profile. Therefore,

the results demonstrated here in our study corroborate the need to evaluate the adjuvant potential of S. skotibergii and I. chordata species in their different stages of development.

The results presented in this study showed that lipid extracts efficiently produced humoral and cellular immune response. The association between macroalgae lipid extracts and the rCP01850 protein elicited a higher antibody production when compared to the negative control and the group immunized with rCP01850 only. The antibody levels elicited in groups G2, G3, G4, G5, and G6 did not differ statistically from the group immunized with the association between rCP01850 and saponin, a commercial adjuvant. Both IFC and IFT led to the highest levels of total IgG, IgG1, and IgG2a antibody production on day 42 but were not significantly different from each other. Furthermore, SFG and SFC were responsible for the increased production of these antibodies when compared to the group immunized with rCP01850 only.

All groups immunized with vaccine formulations containing lipid extracts combined with rCP01850 significantly increased both IgG1 and IgG2a levels. Moreover, eliciting these two IgG subclasses after immunization is an essential step toward concurrently and efficiently producing humoral and cell-mediated immune responses, with each isotype playing a role in eliminating pathogens (Bezerra et al., 2020).

To better characterize whether macroalgae lipid extracts triggered a global immunological response, we evaluated their ability to induce interferon-gamma (IFN-γ) and interleukin 10 (IL-10) production in a splenocyte culture stimulated with rCP01850. The groups immunized with SFG and IFC associated with rCP01850 demonstrated increased IFN-γ production. In turn, all groups immunized with the vaccine formulations containing macroalgae lipid extracts associated with rCP01850 were more efficient in producing IL-10.

IFN-γ is produced by natural killer cells during innate immunity and by CD4+ and CD8+ T cells in the adaptive response phase (Jorgovanovic et al., 2020). This cytokine acts by recognizing and eliminating pathogenic agents, thus being an important player in cellular immune response (Kak et al., 2018). Since *C. pseudotuberculosis* is a facultative intracellular microorganism, the cellular immune response is vital for fighting the infection (Sting et al., 2022).

In turn, IL-10 is produced by Th2 and CD8+ T cells (Gao et al., 2020). This cytokine is important because it regulates several cell types in innate and adaptive immunity and has anti-inflammatory activity (Wei et al., 2020). As previously mentioned, SFG (G3), SFC (G4), IFC (G5), and IFT (G6) significantly increased IL-10 production when compared to G1 and G7. Although protection against intracellular pathogenic agents is better performed by IFN-γ production, producing IL-10 is beneficial since it modulates IFN-γ synthesis. Therefore, IL-10 collaborates positively by avoiding an excessive Th1 response and balancing immune activity during infection (Leal et al., 2018).

Therefore, since one of the greatest challenges in adjuvant research today is finding substances capable of eliciting a humoral immune response (Sena-Lopes et al., 2018) and effectively fighting intracellular microorganisms (Bezerra et al., 2020), our data strongly suggest that using natural compounds, such as fatty acids, can help develop efficient vaccine formulations.

Conclusions

The recombinant protein CP01850, when associated with *Sarcopeltis skottsbergii* and *Iridaea cordata* lipid extracts, promoted a significant humoral immune response and elicited the production of cytokines involved in cellular immunity, consequently rendering a mixed immune response. The vaccine formulations used here effectively protected mice against the infection caused by *Corynebacterium pseudotuberculosis*. Therefore, based on the immune responses induced by marine macroalgae extracts, using them as adjuvants in vaccines against facultative intracellular organisms could be a promising strategy for vaccine development.

Conflict of interests

The authors have no relevant financial or non-financial interests to disclose.

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