Investigating the biological activities of sodium cellobionate produced from cellulosic biomass

Lauren Kovanda,[1] Zhiliang Fan,[2] Xunde Li,[3] and Yanhong Liu[1]
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Abstract

A novel method has been developed to easily hydrolyze cellulose to sodium cellobionate in a filamentous fungus, *Neurospora crassa*. The objectives of this experiment were to investigate the *in vitro* biological activities of sodium cellobionate. Antioxidant activity was evaluated with 3 chemical-based assays, including DPPH radical scavenging assay (**DPPH**), Trolox equivalent antioxidant capacity assay (**TEAC**), and reducing power assay. Antimicrobial activity was determined as minimum inhibitory concentration (**MIC**) that prevented growth of tested bacteria, including 4 gram-negative bacteria (*Escherichia coli* F18 and ATCC 25922, and *Salmonella* Typhimurium ATCC 14028 and a wild strain isolated from cull diary cows in California) and 1 gram-positive bacteria (*Enterococcus faecalis* ATCC 29212). Anti-inflammatory activity was tested by analyzing a proinflammatory cytokine (TNF-α) production with porcine alveolar macrophages that were challenged with lipopolysaccharides. A porcine intestinal epithelial cell line, IPEC-J2, was also used to test the effects of cellobionate on cell proliferation of epithelial cells. The tested doses of sodium cellobionate were 0, 0.04, 0.20, 1.00, 2.00, 4.00, 20.00, and 40.00 mg/mL. All assays were performed with more than 6 replicates, except that MIC assays were performed as triplicate. All data were analyzed by PROC MIXED of SAS. Sodium cellobionate did not have radical scavenging capacity but had weak ferric reducing antioxidant power and Trolox equivalent antioxidant capacity. MIC results revealed that sodium cellobionate did not inhibit the growth of all tested bacteria, indicating it does not have antimicrobial activity within the range of tested doses. Sodium cellobionate did not exhibit anti-inflammatory activities, but significantly enhanced ($P < 0.05$) intestinal epithelial cell proliferation *in vitro* when the dose was lower than 4.00 mg/mL. Results of this experiment indicate that cellobionate has limited biological activities *in vitro*, except that this biomass product could strongly stimulate the proliferation of intestinal epithelial cells. Future research will focus on the potential impacts of sodium cellobionate on intestinal physiology *in vitro* and *in vivo*.

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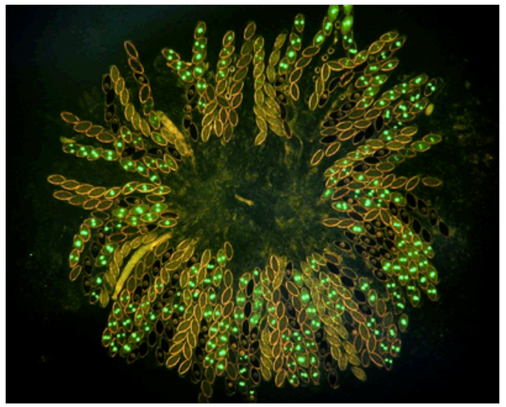
Background

- Microbial fermentation methods are used to produce organic acids.
- Some organic acids have biological properties *in vitro* and *in vivo.*
- Biomass is degraded by microorganisms for biofuel production. One product of cellulosic biomass degradation is an organic acid, cellobionic acid.
- Fungal species, *Neurospora crassa (*Figure 1) readily hydrolyzes cellulose to form cellobiose. It then oxidizes cellobiose to form cellobionate. The salt of the organic acid, sodium cellobionate (SC) is shown in Figure 2.

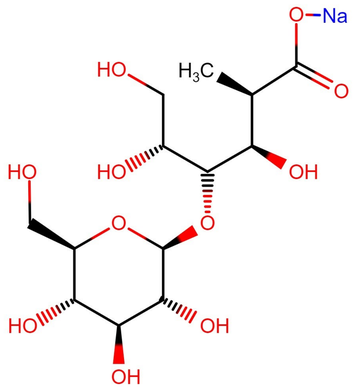
Figure 1. *Neurospora crassa*

Borkovich et al., 2012



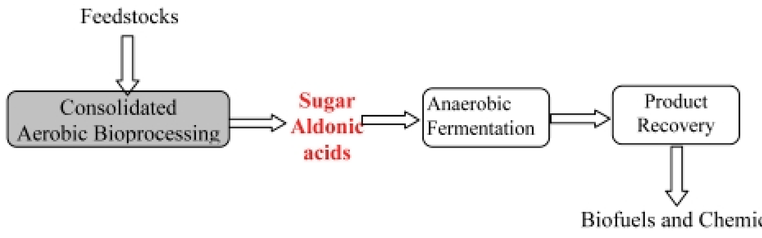
Hurley et al., 2012

Figure 2. Sodium cellobionate (SC)



- Compared with conventional methods for bioprocessing, this pathway (Figure 3) increases the efficiency of degrading cellulosic biomass by hydrolyzing cellulose as well as lignin.

Figure 3. Microbial production of aldonic acids including cellobionic acid



Fan et al., 2012

Objective

Determine the *in vitro* biological activities of sodium cellobionate (**SC**).

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Materials and Methods

- **Anti-inflammatory effects**
  - Porcine alveolar macrophages (**PAMs**) were isolated by bronchial lavage from weaned piglets.
  - Cells were seeded at 1×10^6 cells/mL and cultivated overnight.
  - Cells were then treated, and supernatants were collected after 24h with treatment.
  - Supernatants were analyzed by enzyme-linked immunosorbent assay (**ELISA**) for cellular secretion of tumor-necrosis factor alpha (**TNF-α**).
  - 2×5 factorial experimental design, **n=6**:

		Factor 1: SC level (mg/mL)				
Factor 2:		0.00	0.05	0.20	0.50	2.00
1 µg/mL LPS	+	Trt. 1	Trt. 2	Trt. 3	Trt. 4	Trt. 5
	-	Trt. 6	Trt. 7	Trt. 8	Trt. 9	Trt. 10

- **Antioxidant capacity**
  - Chemical-based assays (Wu et al., 2019)
    - DPPH radical scavenging assay (**DPPH**)
    - Trolox equivalent antioxidant capacity assay (**TEAC**)
    - Reducing power assay
  - Doses: 0.04, 0.20, 1.00, 2.00, 4.00, 20.0, 40.0 mg/mL SC in replicates of 6.

- **Antimicrobial effects**
  - Minimum inhibitory concentration (**MIC**) of bacterial strains was tested using micro-broth dilution method (Stoddard et al., 2008; Li et al., (2015).
  - Tested strains:
    - *Escherichia coli* F18
    - *Escherichia coli* ATCC 25922
    - *Salmonella* Typhimurium ATCC 14028
    - *Salmonella* (wild-type)
    - *Enterococcus faecalis* ATCC 29212
  - Doses: 0.50, 1.00, 5.0, 25.0, 50.0, 100.0, 200.0, 400.0, 500.0, 600.0, 700.0, 800.0, 950.0 mg/mL SC in replicates of 6 or 12.
- **Effects on porcine intestinal cell proliferation**
  - Intestinal porcine epithelial cell line-J2 (IPEC-J2) were utilized.
  - Cells were seeded at 1×10^5 cells/mL and cultivated overnight.
  - Cells were then treated and colorimetric assay using 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (**MTT**) was performed to determine cell viability expressed as percent of control.
  - Doses: 0.00, 0.04, 0.30, 1.00, 2.00, 4.00, 20.0, 40.0 mg/mL SC in replicates of 12.

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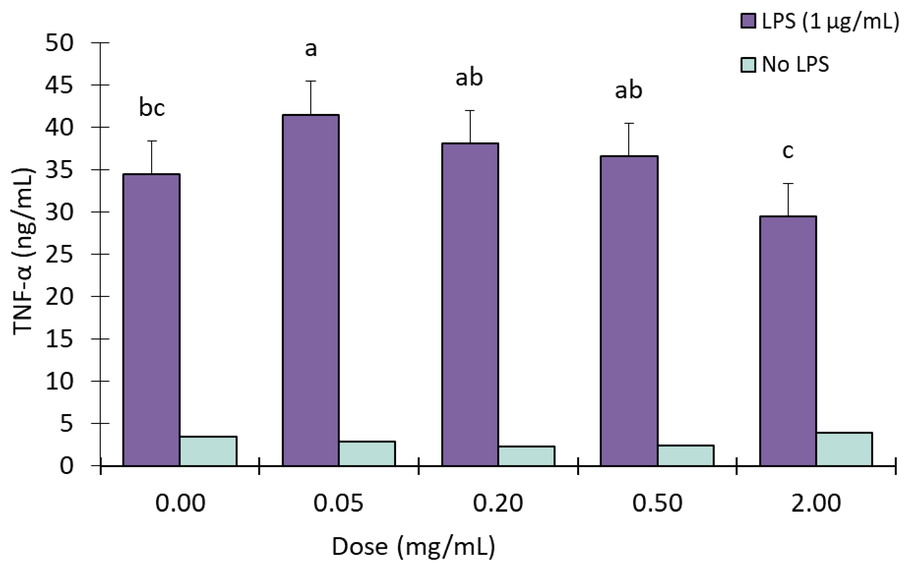
Results

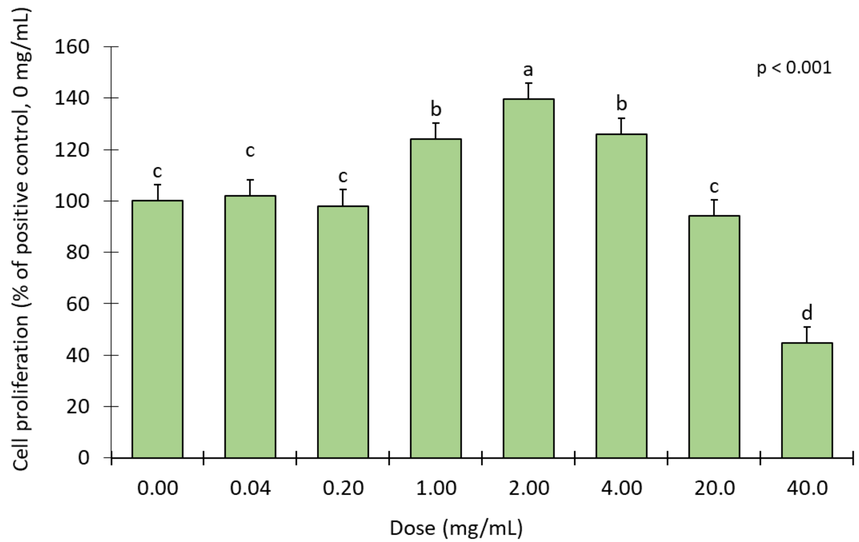
- **Anti-inflammatory effects**
  - SC does not effect the production of tumor TNF-α from porcine alveolar macrophages in the absence or presence of LPS.

Figure 4. TNF-α secretion by porcine alveolar macrophages treated with SC in the absence or presence of LPS. Dose, *P* = 0.1011; LPS, *P* < 0.001; dose*LPS interaction: *P* = 0.0167.



- **Effects on porcine intestinal cell proliferation**
  - SC significantly increased IPEC-J2 cell proliferation at 1.0, 2.0, and 4.0 mg/mL
  - Cytoxicity to IPEC-J2 cells was observed at 40.0 mg/mL.

Figure 5. Cell proliferation of IPEC-J2 treated with SC



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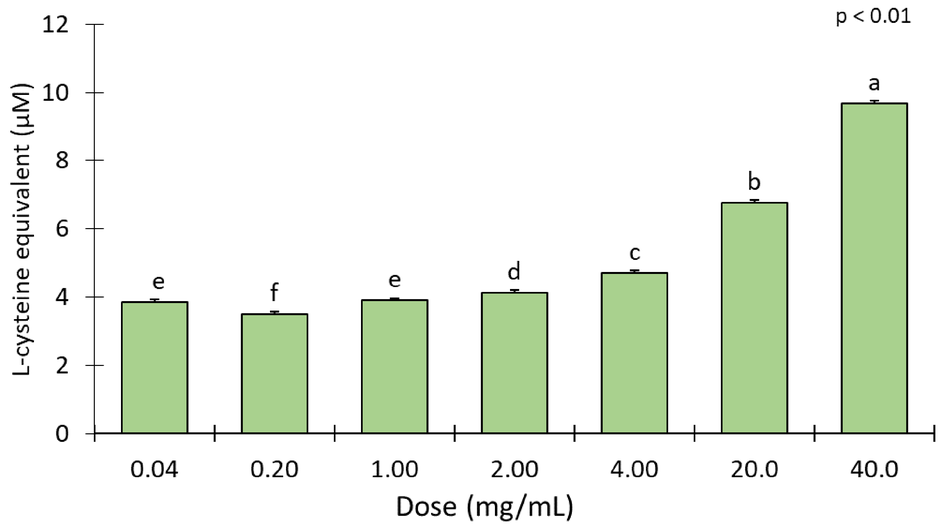
Results

- **Antioxidant capacity**
  - No radical scavenging activity was detected from DPPH assay (data not shown).
  - Weak ferric reducing capacity was observed in TEAC assay (data not shown) and reducing power assay (Figure 6).

Figure 6. Reducing power of SC



- **Antimicrobial effects**
  - No antimicrobial activities were observed against tested strains when SC was tested up to 950 mg/mL (data not shown).

Conclusions

- Sodium cellobionate does not possess antimicrobial, antioxidant, or anti-inflammatory properties.
- Sodium cellobionate stimulates intestinal epithelial cell proliferation *in vitro.*
- Future research may be needed to determine the impact of sodium cellobionate on intestinal integrity *in vivo*.

Acknowledgements and References

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