

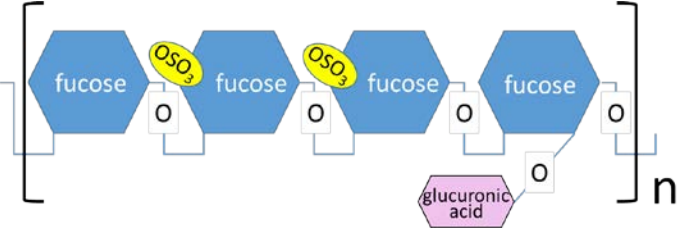


Shunya OKA¹, Masato MIKAMI², Akane IMAI^{3,4} 岡 俊哉¹、三上正人²、今井あかね^{3,4}

¹Dept Biol , ²Dept Microbiol, ³Dept Biochem, Nippon Dent Univ at Niigata, ¹生物, ²微生物, ³生化学, 日本歯科大・新潟
⁴Dept Dent Hygiene Niippon Dent Univ Coll at Niigata ⁴歯科衛生, 日本歯科大・新潟短大

Objective

Fucoidans are sulfated polysaccharides that are found in marine algae.



Fucoidans have been examined for their health benefits and have been reported to exhibit multiple biological activities, such as anticancer, antiulcer, antioxidant, anticoagulant, anti-inflammatory, and immune-modulatory activities. The mechanisms underlying these activities have not yet been completely elucidated.

Methods

- ①Endotoxin-neutralizing activity of fucoidans (LAL assay); After treatment with the Endotoxin Removing Gel (20399, Thermo Fischer Scientific Inc), the ability of fucoidans to bind and neutralize smooth-type LPS was assessed using a quantitative chromogenic *Limulus* ameocyte lysate (LAL) assay [3].
- ②The disk diffusion method [4] was used to examine the antimicrobial and antibacterial effects of fucoidans on oral pathogens. The diameters of the inhibition zones were measured and analyzed by the one-way dispersion method and subjected to multiple comparisons using the Dunnett's test.
- ③The ability of each fucoidan to inhibit the adhesion of *S. mutans* to bovine teeth and porcelain was examined using the MTT assay [5].

Results

- The following results were obtained:
- ①The LAL assay findings suggested the ability of Fucoidans to bind and neutralize LPS.
 - ②Fucoidan derived from Bladderwrack (Sigma crude) exhibited high antimicrobial activity against *C. albicans*, *S. mutans* and *P. gingivalis*. When added to the liquid culture medium, a growth inhibitory effect on *C. albicans* was confirmed.
 - ③Fucoidans also significantly inhibited the adhesion of *S. mutans* to bovine teeth and porcelain.

Materials

Various Fucoidans, crude and purified fucoidan from Bladder wrack (*Fucus vesiculosus*; SIGMA, F5631 and F8190, respectively), sulfated \pm -L-Fucan (Cayman, 9072-19-9), low molecular weight Fucoidan (*Cladosiphon novae-caledoniae*; Daiichi-Sangyo, mixtures of digested and nondigested fractions) and 4% Fucoidan cream (Power fucoidan cream, Daiichi Sangyo) were used in this study. As the oral pathogens, *Candida albicans* (*C. albicans*, JCM1537), *Streptococcus mutans* (*S. mutans*, JCM5705), and *Porphyromonas gingivalis* (*P. gingivalis*, JCM8525) were used. For the MTT assay, alamar-Blue Cell Viability Reagent (Invitrogen, DAL1025) was used.

Conclusion

These results suggest that fucoidans could be useful in oral medicine.

References

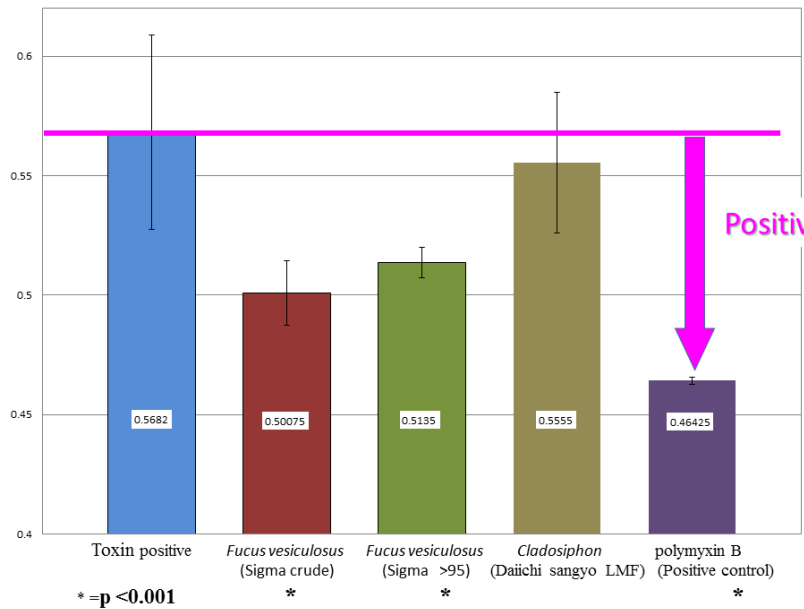
- [1]Tsubura S, *et al.*, *J of Clanio-Max. Diseases*, 2012;1:104-108
- [2] Tsubra S, and Suzuki A, *Dent Open J*. 2017; 5(1): 6-10.
- [3] Taniguchi M, *et al.*, *Peptides*, 2016;75:101-108
- [4] Oka S, *Odontology*. 2018; 106: 46-55.
- [5] Sakuma Y, *et al.*, *Dental Materials J*, 2013;32:585-591

① Endotoxin-neutralizing activity of fucoidans

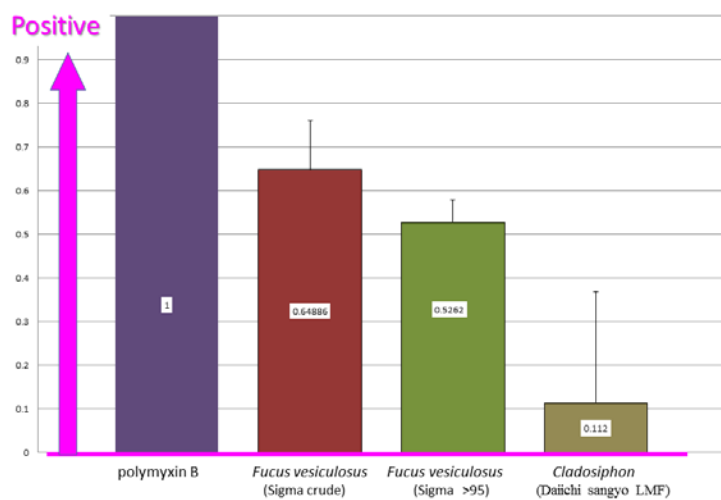
The ability of fucoidans to bind and neutralize smooth-type LPS was assessed using the quantitative chromogenic *Limulus* ameocyte lysate (LAL) assay.

Materials	Mean value OD 405nm, @90min RT	SD	p<0.001
Toxin (60pg)+ LAL (positive cont.)	0.5682	0.0408	
LRW + LAL (negative back ground)	0.1146	0.0177	*
Bladderwrack 0.1μg/μℓ (Sigma, F5631, crude)	0.5001	0.0136	*
Bladderwrack 0.1μg/μℓ (Sigma, F8190, >95)	0.5135	0.0064	*
Namacystus 0.1μg/μℓ (Daiichi sangyo, LMF)	0.5555	0.0294	
Polymyxin B 10μU/μℓ (neutralize positive cont.)	0.4643	0.0015	*

The LAL assay findings suggested the ability of Fucoidans to bind and neutralize endotoxin, similar to polymyxin-B.



The quantifiable data obtained were analyzed by f-test and t-test. Statistical significance was set at P < 0.001

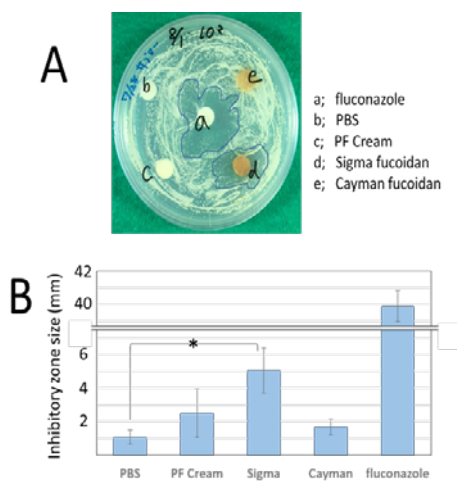


The endotoxin neutralizing activity of each Fucoidan sample (0.1μg/μℓ) was expressed relative to that of polymyxin-B (10μU/μℓ).

② Antimicrobial activity of Fucoidans

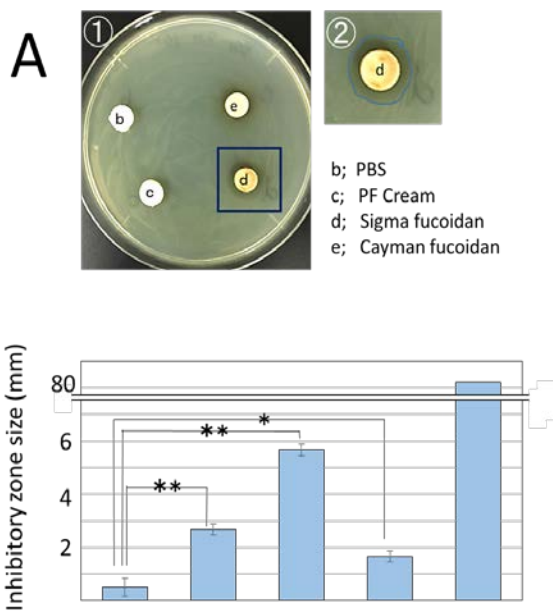
Fucoidan derived from Bladderwrack (SIGMA, crude, F5631) significantly inhibited proliferation of the oral pathogens examined.

C. albicans



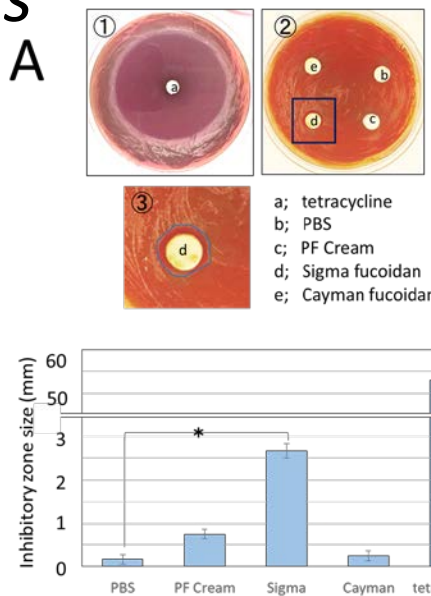
- A: Typical inhibitory zone against *C. albicans*.
a: Fluconazole was used as a positive control. d: A inhibitory zone around the Sigma fucoidan disc; although the zone was an unstructured circle.
B: Size of the inhibitory zone against *C. albicans**, P < 0.05.
C: Spot test of *C. albicans* growth. Fucoidan or fluconazole (positive control) were added to overnight culture of *C. albicans* (500 cells/ml), and diluents were spotted on the PYG agar plate and incubated at 37°C overnight. PF cream and Sigma fucoidan inhibited the proliferation of *C. albicans*.

S. mutans



- A: ①Typical inhibitory zone against *S. mutans*. Penicillin was used as a positive control (data not shown). ②Magnified view of the blue box on the left..
d: A clear inhibitory zone around the Sigma fucoidan disc.
B: Size of the inhibitory zone against *S. mutans*. **, P < 0.01. *, P < 0.05.

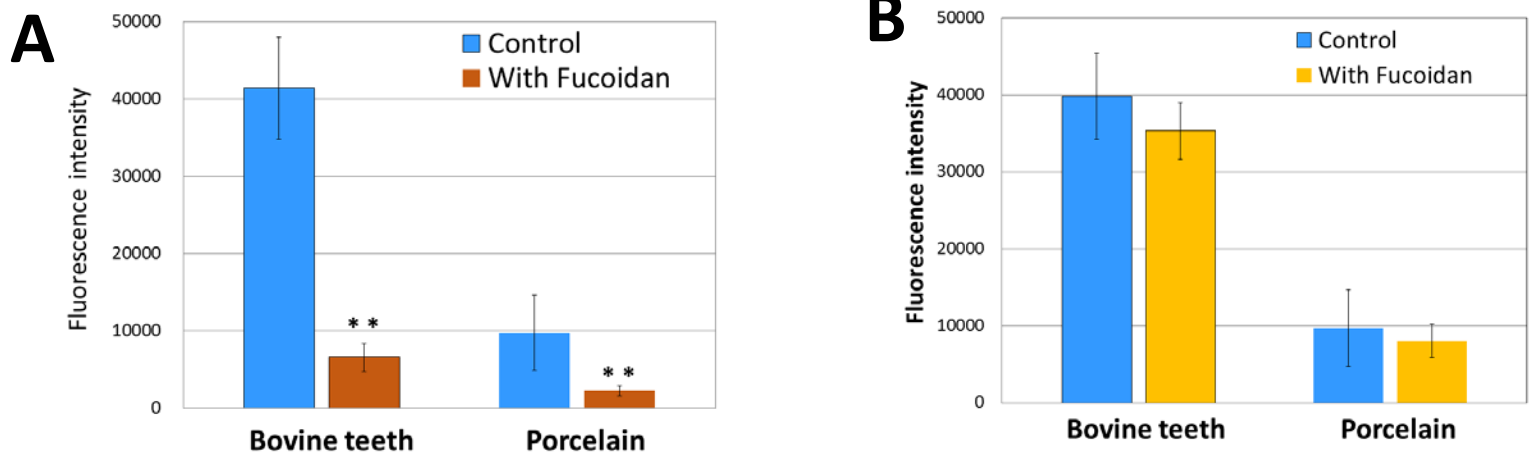
P. gingivalis



- A: Typical inhibitory zone against *P. gingivalis*.
a: Tetracycline was used as a positive control. ③shows a magnified view of the blue box on the right.
d: Clear inhibitory zone around the Sigma fucoidan disc.
B: Size of the inhibitory-zone against *P. gingivalis*. **, P < 0.01. *, P < 0.05.

③ Inhibition of *S. mutans* adhesion by Fucoidans

The ability to inhibit adhesion of *S. mutans* to bovine teeth and porcelain was examined using the MTT assay.



- A: Inhibition of *S. mutans* adhesion by Bladder wrack SIGMA crude(*: P < 0.01)
B: Inhibition of *S. mutans* adhesion by Bladder wrack SIGMA >95%

Fucoidan from Bladderwrack (SIGMA crude, F5631, 50μg/μℓ) significantly inhibited the adhesion of *S. mutans* to bovine teeth and porcelain.

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Conflict of Interest

The authors declare no conflict of interest.