

Research Article

Investigation of Anti-bacterial Activity against Food-borne Pathogens among Korean Domestic Algae

¹Ki-hyo Jang and ²Je-hyuk Lee

¹Department of Food and Nutrition, Kangwon National University, Samcheok, Gangwon, 245-905, Korea

²Department of Food and Nutrition, Kongju National University, Yesan, Chungnam, 340-702, Korea

Abstract: The aim of this study is to explore algal species with anti-bacterial activity against six food-borne pathogens. Among 51 marine algae, *Laurencia okamurae* Yamada and *Dictyopteris undulata* Holmes was elucidated to have a potent anti-bacterial activity against food-borne pathogens. *Laurencia okamurae* Yamada showed the clear zone around agar well on *B. cereus*, *S. aureus* and *L. monocytogenes*-spreading agar plate. *Dictyopteris undulata* Holmes had the anti-bacterial activity against *S. choleraesuis*, *B. cereus*, *S. aureus* and *L. monocytogenes* on bacterial spreading agar plates. Antibacterial activity of *L. okamurae* Yamada and *D. undulata* Holmes had specifically susceptibility for *B. cereus*, *S. aureus* and *L. monocytogenes* and were superior to streptomycin, the authentic antibiotics. It is anticipated that new food preservatives can be explored and developed on the basis of this study.

Keywords: Algae, anti-bacterial activity, *Dictyopteris undulata* Holmes, food-borne pathogens, *Laurencia okamurae* Yamada

INTRODUCTION

Foodborne diseases are still a major concern in the world. In 2005, it was reported that 1.8 million people died from diarrhoeal diseases, largely attributable to contaminated food and drinking water by World Health Organization (WHO). This is not just an underdeveloped world problem. About 76 million cases of food-borne diseases, resulting in 325,000 hospitalizations and 5000 deaths, are estimated to occur each year in the United States of America (USA) alone (Mead *et al.*, 1999). According to the reports regarding foodborne microorganisms outbreak, *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* were found in eggs, beef, chicken and dairy products in the U.S.A. and the European Union (Tauxe, 2002; Greig and Ravel, 2009).

Due to the worldwide awareness on chemical preservatives, the food industry is now reflected by the consumer opinions for safer additives and thus is focusing on natural safe preservatives (Dillon and Board, 1994). Some plant materials are used as natural antimicrobials in food systems, as well as to prevent the growth of foodborne bacteria and molds resulting in shelf-life extension of processed foods. Fruits, vegetables, grains and food constituents can be contaminated by various microorganisms and their hazardous toxic metabolites. Enterotoxins produced by *Escherichia coli*, *S. pyogenes*, *Salmonella*, *Yersinia* and

Clostridium species are responsible for toxicity in the intestinal tract causing vomiting, diarrhea, etc. Moreover, microorganisms are also associated with food spoilage causing economical loss (Harris, 1988; Rocourt *et al.*, 2003). Although most healthy humans are not significantly affected by low doses of the bacteria, the pathogen can be more harmful for people with weak immune systems or during pregnancy. Among severe infections, even listeriosis by *Listeria* sp., has been associated with high mortality rate (Datta, 2003).

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae (Lindequist and Schweder, 2001; Newman *et al.*, 2003). There are numerous reports concerning the inhibiting activities from macro-algae against human pathogens, fungi and yeasts (Sridhar and Vidyavathi, 1991; Mahasneh *et al.*, 1995; De Val *et al.*, 2001; Liao *et al.*, 2003). It was reported that bromophenol, dolabellane derivatives, phloroglycin and hydroquinone derivatives were isolated as anti-bacterial compounds from red algae, Rhodomelaceae, *Dictyota dichotoma*, *Fucus vesiculosus* and *Dictyopteris zonarioides* (Choi *et al.*, 2000; Amico *et al.*, 1980). However, little attention has been given to the research

Corresponding Author: Je-Hyuk Lee, Department of Food and Nutrition, Kongju National University, Yesan, Chungnam, 340-702, Korea

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Table 1: Agar diffusion susceptibility of foodborne pathogens to Korea domestic algae extract

| Sea algae | JBRI coll. No. ^a | Clear zone (mm) ^b | | | | | |
|--|-----------------------------|------------------------------|--------------------|------------------|--------------------|------------------|-------------------------|
| | | <i>S. choleraesuis</i> | <i>S. enterica</i> | <i>B. cereus</i> | <i>B. subtilis</i> | <i>S. aureus</i> | <i>L. monocytogenes</i> |
| <i>Endarachne binghamiae</i> J. Agardh | 10173 | - ^c | 0.5±0.1 | - | - | 0.5±0.1 | - |
| <i>Dictyopteris prolifera</i> (Okamura) Okamura | 10242 | 0.5±0.1 | - | - | - | 0.5±0.1 | 0.5±0.1 |
| <i>Sargassum nipponium</i> Yendo | 10250 | - | - | 0.5±0.1 | - | 0.5±0.1 | 0.5±0.1 |
| <i>Ulva pertusa</i> Kjellman | 10253 | - | - | - | - | 0.5±0.1 | 0.5±0.1 |
| <i>Dictyopteris divaricata</i> (Okamura) Okamura | 10254 | - | - | - | - | 0.5±0.1 | - |
| <i>Dictyota asiatica</i> Hwang et al. | 10255 | - | - | - | - | 0.5±0.1 | 0.5±0.1 |
| <i>Padina arborescens</i> Holmes | 10258 | 0.5±0.1 | - | 0.5±0.1 | 0.5±0.1 | 1 | - |
| <i>Sargassum muticum</i> (Yendo) Fensholt | 10261 | 0.5±0.1 | - | 0.5±0.1 | 0.5±0.1 | 0.5±0.1 | - |
| <i>Myelophycus simplex</i> (Harvey) Papenfuss | 10262 | - | - | 1 | 0.5±0.1 | - | - |
| <i>Chondria crassicaulis</i> Harvey | 10264 | - | - | 0.5±0.1 | - | - | 0.5±0.1 |
| <i>Dictyopteris pacifica</i> (Yendo) Hwang et Kim comb. Nov. | 10265 | 0.5±0.1 | - | 1 | - | 0.5±0.1 | - |
| <i>Lomentaria hakodatensis</i> Yendo | 10266 | 0.5±0.1 | 0.5±0.1 | 1 | - | 1 | 0.5±0.1 |
| <i>Sargassum</i> sp. | 10269 | 0.5±0.1 | - | 1 | - | - | 0.5±0.1 |
| <i>Chondracanthus tenellus</i> (Harvey) Homm. in Homm. | 10270 | 0.5±0.1 | - | 1 | - | 0.5±0.1 | - |
| <i>Dictyopteris prolifera</i> (Okamura) Okamura | 10271 | 0.5±0.1 | - | 1 | - | 1 | - |
| <i>Prionitis cornea</i> (Okamura) Dawson | 10272 | 0.5±0.1 | - | 1 | - | - | 0.5±0.1 |
| <i>Ishige okamurae</i> Yendo | 10276 | - | - | 0.5±0.1 | - | 0.5±0.1 | - |
| <i>Bonnemaisonia hamifera</i> Hariot | 10278 | 0.5±0.1 | - | - | - | 0.5±0.1 | 0.5±0.1 |
| <i>Laurencia intricata</i> Lamourx | 10280 | - | - | 0.5±0.1 | - | 0.5±0.1 | 1.0±0.5 |
| <i>Sargassum fulvellum</i> (Turner) C. Agardh | 10281 | - | - | 0.5±0.1 | - | - | - |
| <i>Ecklonia cava</i> Kjellman | 10282 | 0.5±0.1 | - | 0.5±0.1 | - | 0.5±0.1 | - |
| <i>Scytosiphon lomentaria</i> (Lyngbye) Link | 10350 | 0.5±0.1 | - | 1 | - | 0.5±0.1 | - |
| <i>Laurencia intermedia</i> Lamouroux | 10351 | - | - | - | - | 0.5±0.1 | - |
| <i>Grateloupia filicina</i> (Lamouroux) C. Agardh | 10352 | - | - | 1 | - | 0.5±0.1 | - |
| <i>Callophyllis crispata</i> Okamura | 10353 | 0.5±0.1 | - | 0.5±0.1 | - | - | - |
| <i>Laurencia okamurae</i> Yamada | 10354 | - | - | 4 | - | 3 | 2.6±0.5 |
| <i>Colpomenia sinusa</i> (Mertens ex Roth) Derbes et Solier in Castagne | 10355 | - | - | - | - | 0.5±0.1 | - |
| <i>Polyopes affinis</i> (Harvey) Kawaguchi et Wang in Kawaguchi, Wang, Horiguchi | 10359 | - | - | 0.5±0.1 | - | 0.5±0.1 | 0.5±0.1 |
| <i>Lethesia difformis</i> (Linnaeus) Areschoug | 10363 | 0.5±0.1 | - | 0.5±0.1 | - | - | - |
| <i>Chondrus ocellatus</i> Holmes | 10364 | - | - | 0.5±0.1 | - | - | - |
| <i>Polysiphonia morrowii</i> Harvey | 10365 | - | - | 0.5±0.1 | - | - | 0.5±0.1 |
| <i>Hydroclathrus clathratus</i> (C. Agardh) Howe | 10366 | - | 0.5±0.1 | 0.5±0.1 | - | 2 | - |
| <i>Sargassum thunbergii</i> (Mertens ex Roth) Kuntze | 10367 | 0.5±0.1 | - | 1.5 | - | 0.5±0.1 | - |
| <i>Hizikia fusiformis</i> (Harvey) Okamura | 10495 | - | - | 1.5 | - | 0.5±0.1 | - |
| <i>Grateloupia elliptica</i> Holmes | 10513 | 0.5±0.1 | - | 1.5 | - | 0.5±0.1 | - |
| <i>Scytosiphon gracilis</i> Kogame | 10517 | 0.5±0.1 | - | 1.5 | - | 1 | - |
| <i>Sargassum micracanthum</i> (Kuntze) Endlicher | 10519 | 0.5±0.1 | - | - | - | 0.5±0.1 | - |
| <i>Chondrus crispus</i> Stackhouse | 10520 | - | - | 2 | - | 0.5±0.1 | 0.5±0.1 |
| <i>Gloiopertis complanta</i> (Harvey) Yamada | 10527 | - | - | 2 | - | 0.5±0.1 | - |
| <i>Gloiopertis furcata</i> (Postels et Ruprecht) J. Agardh | 10530 | - | - | 2 | - | 0.5±0.1 | 0.5±0.1 |
| <i>Grateloupia lanceolata</i> (Okamura) Kawaguchi | 10559 | - | 0.5±0.1 | 0.5±0.1 | - | 2 | - |
| <i>Gelidium amansii</i> (Lam.) Lamouroux | 10560 | - | - | - | - | 0.5±0.1 | - |
| <i>Dictyopteris undulata</i> Holmes | 10656 | 0.5±0.1 | - | 5 | - | 4 | 9.0±1.0 |
| <i>Sargassum micracanthum</i> (Kuntze) Endlicher | 10682 | - | 0.5±0.1 | 2 | - | 1 | - |
| <i>Ecklonia stolonifera</i> Okamura | 10692 | - | - | 2 | - | - | - |
| <i>Sargassum confusum</i> C. Agardh f. <i>validum</i> Yendo | 10696 | - | 0.5±0.1 | - | - | - | 0.5±0.1 |
| <i>Sargassum sagamianum</i> Yendo | 10697 | - | 0.5±0.1 | 2 | - | - | 0.5±0.1 |

Table 1: (Continue)

| Sea algae | JBRI coll. No. ^a | Clear zone (mm) | | | | | |
|--|-----------------------------|------------------------|--------------------|------------------|--------------------|------------------|-------------------------|
| | | <i>S. choleraesuis</i> | <i>S. enterica</i> | <i>B. cereus</i> | <i>B. subtilis</i> | <i>S. aureus</i> | <i>L. monocytogenes</i> |
| <i>Gracilaria verrucosa</i> (Hudson) Papenfuss | 10698 | - | - | 1 | - | 0.5±0.1 | - |
| <i>Sargassum horneri</i> (Turner) C. Agardh | 20040 | 0.5±0.1 | 0.5±0.1 | - | - | 1 | 0.5±0.1 |
| <i>Sargassm patens</i> C. Agardh | 10066 | 0.5±0.1 | - | 1 | - | 0.5±0.1 | - |
| <i>Sargassum macrocarpum</i> C. Agarch | 10069 | - | 0.5±0.1 | 1.5 | - | 1 | - |
| Streptomycin | | 5.0±1.0 | 6.93±0.8 | 10.0±1.3 | 6.25±1.9 | 3.93±1.2 | 4.8±1.0 |

The antimicrobial activities were determined by agar-well diffusion assay; After 50 µL of each bacterial suspension (OD600 = 0.2-0.3, approximately 1×10⁷ cfu/mL) were spread uniformly onto the surface of each agar plate (1.5%); The well was cut into the set agar using a sterile cork-borer (5 mm diameter); Sea weeds extracts (10 µg/well) were dispensed into the wells; After 24 h incubation, the inhibition zones around wells were measured in mm using a caliper; ^a: Jeju Biodiversity Research Institute (JBRI) collection number; ^b: Semi-diameter (mm) of clear zone, expressed by average±standard deviation from triplicate trials; ^c -: Not detected

of Korean domestic algae for noble anti-foodborne pathogens.

In this report, we focused on the studies for the investigation of anti-bacterial activities of Korean domestic algae against foodborne pathogens, which were highly issued recently in South Korea. It is anticipated that new food preservatives can be explored and developed on the basis of this study.

MATERIALS AND METHODS

Sea weeds materials: All sea weed was collected in sea near Jeju island Korea, authenticated by Jeju Biodiversity Research Institute (JBRI), Jeju, Korea and deposited as the voucher specimen (Table 1) to JBRI.

Each sea weed was washed, dried by freeze drier and crushed. Crushed weeds were extracted with 70% methanol (in water) at room temperature. Extracts were filtered through Whatman No. 1 filter paper and were concentrated by evaporator under reduced pressure. Sea weed-extract was redissolved in DMSO to 100 mg/mL, stored at -20°C and used it as a stock.

Microorganisms and growth conditions: Four gram-positive (*S. aureus*, *B. cereus*, *B. subtilis* and *Listeria monocytogenes*) and two gram-negative foodborne pathogens (*S. choleraesuis* and *S. enterica*) were used in the experiments. *S. aureus* American Tissue Culture Collection (ATCC) 6538P, *S. choleraesuis* ATCC 13312 and *B. cereus* ATCC 14579 were purchased from ATCC and *L. monocytogenes* KCTC 3710, *B. subtilis* KCTC 3726 and *S. enterica* KCTC 12400 were obtained from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea). Stock cultures of bacteria were kept in 35% glycerol phosphate buffered saline at -70°C. All bacterial strains were cultured by inoculating 100 mL of the thawed microbial stock suspensions into 5 mL Nutrient Broth (Difco, Detroit, MI) in 100 rpm shaking incubator at 37°C for 24 h in aerobic condition.

Agar-well diffusion assay: The antimicrobial activities of sea weeds extracts were evaluated by agar-well diffusion assay. The assay was carried out according to the method (Owen and Palombo, 2007) of with some

modifications. After 50 mL of each bacterial suspension (OD600 = 0.2-0.3, approximately 1×10⁷ cfu/mL) were spread uniformly onto the surface of each agar plate (1.5%), the well was cut into the set agar using a sterile cork-borer (5 mm diameter). Sea weeds extracts (10 µg/well) were dispensed into the wells. Agar plates were left at room temperature for 30 min to allow the liquid to diffuse into the agar before overnight incubation at 37°C. All assays were carried out in triplicate. A clear zone of the inhibited microbial growth surrounded substances was considered positive exhibiting antibacterial properties. The solvent system used for the preparation of sea weeds extract samples was used for blank well. After incubation, the inhibition zones around wells were measured in mm using a caliper (Fazeli *et al.*, 2007). Ampicillin and streptomycin (10 µg/well) were used as positive controls.

Statistical assay: All result was expressed as mean±Standard Deviation (S.D.) and were analyzed using one way Analysis of Variance (ANOVA) and Dunnett's multiple comparison test for individual comparisons. Results were considered statistically significant when p-values were p<0.05.

RESULTS AND DISCUSSION

Anti-bacterial activity of marine algal extracts against food-borne pathogens: The huge number of marine algal species inhabit in the coast of Jeju-island, Korea (Lee, 2008). Fifty one algae collected from seashore of Jeju-island were investigated for anti-bacterial activity against food-borne pathogens. The aim of this study is to explore algal species with anti-bacterial activity against six food-borne pathogens issued in Korea recently. Antibacterial activity of marine algae was determined by agar well diffusion assay. Control treatment (DMSO) showed no clear zone around agar-wells against any of pathogens (Fig. 1).

Among 51 marine algae, *Laurencia okamurae* Yamada and *Dictyopteris undulata* Holmes was elucidated to have a potent anti-bacterial activity against food-borne pathogens (Fig. 1 and Table 1).

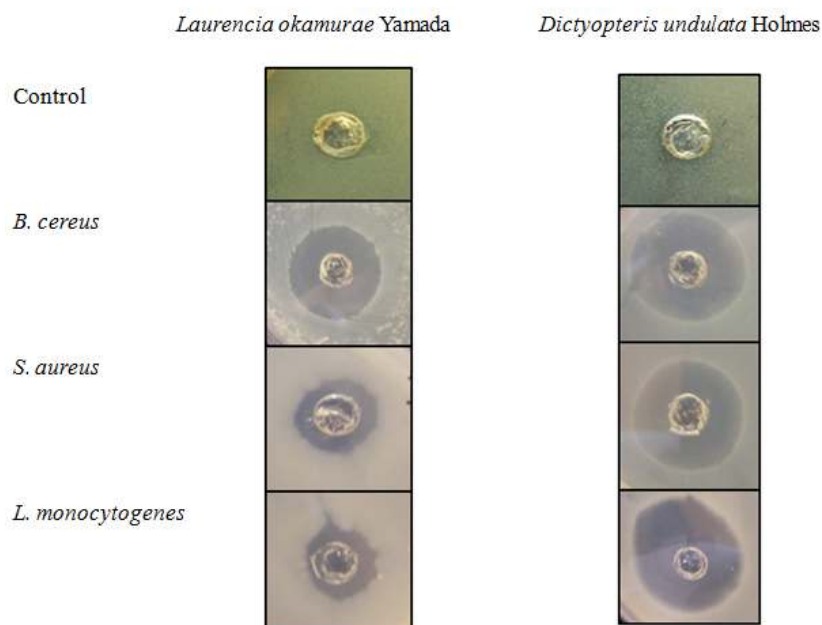


Fig. 1: Anti-bacterial activities of *Laurencia okamurae* Yamada and *Dictyopteris undulata* Holmes against food-borne pathogens. After 50 μ L of *B. cereus*, *L. monocytogenes* and *S. aureus* culture suspension (OD600 = 0.2-0.3, approximately 1×10^7 cfu/mL) were spread uniformly onto the surface of each agar plate (1.5%), the well was cut into the set agar using a sterile cork-borer (5 mm diameter). The Solvent system (DMSO) used for the preparation of extract samples was used for control. Extracts (10 μ g/well) of *Laurencia okamurae* Yamada and *Dictyopteris undulata* Holmes were dispensed into the wells. After 24 h incubation, the inhibition zones around wells were measured

Laurencia okamurae Yamada showed the inhibition zone of approximately 4.0 ± 0.2 mm around agar well on *B. cereus*-spreading agar plate. In addition, *L. okamurae* Yamada had antibacterial activities against *S. aureus* and *L. monocytogenes* with approximately 3.0 ± 0.3 and 2.6 ± 0.5 mm, respectively. Another marine algae, *Dictyopteris undulata* Holmes had the antibacterial activity against *S. choleraesuis* with approximately 0.5 ± 0.1 mm, *B. cereus* with approximately 5.0 ± 0.2 mm, *S. aureus* with approximately 4.0 ± 0.3 mm and *L. monocytogenes* with approximately 9.0 ± 1.0 mm clear zones on bacterial spreading agar plates. Antibacterial activity of *L. okamurae* Yamada and *D. undulata* Holmes had specifically susceptibility for *B. cereus*, *S. aureus* and *L. monocytogenes* and were superior to streptomycin, the authentic antibiotics. However, streptomycin, which is a broad-spectrum antibiotics, showed the clear zone for all tested food-borne pathogens.

Great attention has been focusing on the marine seaweeds as potential and promising sources of bioactive compounds in recent years. Seaweeds are able to produce a great variety of secondary metabolites with versatile activities, such as antiviral, anthelmintic, antifungal and antibacterial activities (Lindequist and Schweder, 2001; Newman *et al.*, 2003). Red algal genus *Laurencia* (Rhodomelaceae, Ceramiales) produces the numerous specialized secondary metabolites with diverse structural features. Its three

major classes, which are sesquiterpenes, diterpenes and acetylenes, have not yet been encountered in other terrestrial organisms (Vairappan, 2003; Erikson, 1983; Fenical, 1975). Most of these metabolites are characterized by the presence of halogen atoms in their chemical formula. Bromine-containing compounds are more abundant than either chlorine- or iodine-containing ones. Halogenated compounds and metabolites from *Laurencia* sp., have been investigated to be due to antimicrobial, insecticidal, cytotoxic and feeding-deterrent activities (Konig and Wright, 1997; Hay *et al.*, 1987).

Due to increases in bacterial resistance against commercial antibiotics, there is a growing need, not only in human but also in veterinary medicine, for new antibacterial compounds that are active against pathogenic bacteria. It is reasonable to suggest some of these compounds as seaweed's defense chemicals to protect themselves against pathogens and survive in the marine ecosystem to potential anti-bacterial agents. It was reported that metabolites isolated from Malaysian *Laurencia majuscula* could inhibit its surface bacteria, suggesting the presence of an inherently available antibacterial defense mechanism (Vairappan *et al.*, 2001). Some halogenated metabolites from *Laurencia* have been shown to have antibacterial activity against terrestrial bacteria (Vairappan *et al.*, 2001), thus suggesting that halogenated compounds have the potential to function as chemical defense substances

against pathogenic bacteria. Isolated metabolites including halogenated metabolites, such as 10-acetoxyangasiol, aplysiadiol, cupalaurenol, 10-acetoxyangasiol, aplysiadiol, cupalaurenol and chamigrane epoxide, exhibited potent antibacterial activities against clinical bacteria, *Staphylococcus aureus*, *Staphylococcus* sp., *Streptococcus pyogenes*, *Salmonella* sp. and *Vibrio cholerae*. To date, there has been minimal research regarding the anti-bacterial activity against food-borne pathogens.

Brown algae, *Phaeophyceae*, are a class of almost exclusively marine organisms that have been explored for the bioactivity potential of its metabolic products, especially those of the representative family Dictyotaceae. Dictyotaceae natural products include a rich production of terpenoids of different origins and are therefore important in studies of metabolites of marine origin (Vallim *et al.*, 2010). The most bioactive products have three or four isoprenoid units, corresponding to the sesquiterpenes and diterpenes, respectively. These two classes of terpenoids can have many different structures and variants, including the addition of halogen atoms or fragments from other biogenic pathways. Halogenated sesquiterpenes in which a phloroglucinol unit were reported in *Dictyopteris divaricata* (Ji *et al.*, 2009) and *D. undulata* (Song *et al.*, 2004a). The sesquiterpenes of these genera are known to exhibit activity as an inhibitor of herbivores and antifungal, cytotoxic, antibiotic and ichthyotoxic activities. In addition, the cytotoxic activities were evaluated in several human tumor cell lines for sesquiterpenes from *Dictyopteris* (Fenical *et al.*, 1973) and also the antifungal activity was found only for sesquiterpenes from *Dictyopteris*. Therefore a polysaccharide-rich extracts from *Dictyopteris* sp., are expected to exhibit assorted biological activities, including anticoagulant, antiproliferative and antioxidant activities.

CONCLUSION

This result is able to provide a fundamental data of screening for marine algae with anti-bacterial activity against food-borne pathogens. *Laurencia okamurae* Yamada and *Dictyopteris undulata* Holmes was investigated to have a potent anti-bacterial activity against food-borne pathogens. And it is expected to be possible to develop a novel and natural food preservative using extracts of *Laurencia okamurae* Yamada and *Dictyopteris undulata* Holmes.

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