



Effects of alkyl gallates, fatty acids, and acylglycerols on the growth of the psychrotolerant bacterium *Sporosarcina* sp. S92h

Kentaro Tsuda^{a,d}, Hideaki Nagano^b, Ryota Nakatsuji^a, Michiki Takeuchi^{b,d}, Akinori Ando^{c,d}, Jun Shima^{e,1}, Jun Ogawa^{c,d,*}

^a Food Development Laboratories, Nippon Shinyaku Co. Ltd, Kyoto 601-8550, Japan

^b Industrial Microbiology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

^c Research Unit for Physiological Chemistry, Kyoto University, Kyoto 606-8502, Japan

^d Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

^e Research Division of Microbial Sciences, Kyoto University, Kyoto 606-8502, Japan

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ABSTRACT

Sporosarcina species are psychrotolerant endospore-forming bacteria that are predominantly isolated from processed minced fish meat (surimi). Their levels should be controlled to maintain the quality of surimi-based food products. Our previous study suggested that the fatty acid composition of the cell membrane is an important factor for the growth of *Sporosarcina* species under cold conditions. In this study, we examined the effects of alkyl gallates, fatty acids, and acylglycerols on the growth of *Sporosarcina* sp. S92h in TSB medium at 10 °C and 30 °C. The results illustrated that the alkyl gallates isoamyl gallate, octyl gallate, and lauryl gallate; the fatty acids oleic acid, linoleic acid, and α -linolenic acid; and the acylglycerol monolaurin inhibited the growth of *Sporosarcina* sp. S92h for 10 days at 10 °C at a concentration of 0.2 mM. We found that compounds that exerted significant growth-inhibitory effects have similar molecular weights (range, 240.3–338.4) and ACD/LogP values (range, 3.48–7.70). These data will be useful for exploring more effective compounds to inhibit the growth of *Sporosarcina* species and contribute to quality control for surimi-based foods.

1. Introduction

Psychrotolerant endospore-forming bacteria are important contaminants in various processed foods because they can survive pasteurization and subsequently grow during refrigeration. Previously, we reported that several *Sporosarcina* species were predominantly isolated from minced fish meat (surimi) as psychrotolerant endospore-forming bacteria, and we suggested that they should be controlled to maintain the quality of surimi-based food products (Tsuda et al., 2015). However, there are few reports regarding food additives for inhibiting bacterial growth in food products.

An important explanation for bacterial growth in cold environments is modulation of the fatty acid composition of the cellular membrane in response to cold temperatures (Zhang and Rock, 2008). Modulation of the membrane fatty acid composition is considered necessary for maintaining its fluidity. Our previous study suggested that the contents of both branched-chain (BCFAs) and unsaturated fatty acids (UFAs) are important factors concerning the growth of *Sporosarcina* strains under

cold conditions (Tsuda et al., 2017), as well as *Listeria monocytogenes* (Annou et al., 1997) and *Bacillus cereus* (de Sarrau et al., 2012). BCFAs, including *iso*- and *anteiso*-fatty acids, are major components of lipids in several bacteria, such as *Bacillus* (Kaneda, 1977), *Listeria* (Feresu and Jones, 1988), and *Sporosarcina* (Yoon et al., 2001) species. In these bacteria, BCFAs are produced from their corresponding branched-chain amino acids (BCAAs), including valine, leucine, and isoleucine, via transamination and decarboxylation reactions and by the type II fatty acid synthase cycle (Kaneda, 1991). In accordance with the fatty acid biosynthesis pathway, BCAA supplementation could modify BCFA and UFA content and control the growth of *Sporosarcina* species at cold temperatures (Tsuda et al., 2017). It is likely that the cold adaptation of *Sporosarcina* species could be affected by exogenous compounds that interact with the bacterial cell membrane.

We are interested in developing preservatives that can inhibit the growth of psychrotolerant bacteria, especially *Sporosarcina* species, to improve the quality of refrigerated food products. In this study, we tested the effects of hydrophobic compounds that potentially interact

* Corresponding author at: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan.

E-mail address: ogawa@kais.kyoto-u.ac.jp (J. Ogawa).

¹ Present address: Faculty of Agriculture, Ryukoku University, Shiga 520-2194, Japan.

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Table 1

Physicochemical properties of the tested compounds and specific growth rates of *Sporosarcina* sp. S92h at 30 °C and 10 °C in TSB medium with the compounds at final concentration of 0.2 mM.

Compounds (abbreviation)	MW	ACD/LogP	μ_{\max} (h ⁻¹)		Lag time (day)*	
			30 °C	10 °C	30 °C	10 °C
Controls						
Blank			0.789	0.140	0.38	2.33
1.0% Acetone			0.759	0.140	0.39	2.34
0.5% Ethanol			0.798	0.137	0.36	2.32
Alkyl gallates						
Methyl gallate (MG)	184.2	1.54	0.745 ^w	0.158 ^w	0.44	2.04
Ethyl gallate (EG)	198.2	2.07	0.725 ^w	0.134 ^w	0.46	2.11
Propyl gallate (PG)	212.2	2.60	0.686 ^w	0.106 ^w	0.50	2.56
Butyl gallate (BG)	226.2	3.13	0.552 ^w	0.096 ^w	0.56	2.79
Isoamyl gallate (IG)	240.3	3.48	0.384 ^w	NG ^w	0.67	NG ^a
Octyl gallate (OG)	282.3	5.26	NG ^a	NG ^a	NG ^a	NG ^a
Lauryl gallate (LG)	338.4	7.38	NG ^a	NG ^a	NG ^a	NG ^a
Stearyl gallate (SG)	422.6	10.57	0.127 ^a	0.131 ^a	2.53	2.39
Free fatty acids						
Stearic acid (C18:0)	284.5	8.22	0.514 ^e	0.108 ^e	0.40	2.17
Oleic acid (C18:1)	282.5	7.70	NG ^e	NG ^e	NG ^a	NG ^a
Linoleic acid (C18:2)	280.5	7.18	NG ^e	NG ^e	NG ^a	NG ^a
α -Linolenic acid (C18:3)	278.4	6.50	NG ^e	NG ^e	NG ^a	NG ^a
Monoacylglycerols						
Monocaprylin (C8MG)	218.3	1.91	0.472 ^e	0.110 ^e	1.93	5.52
Monocaprin (C10MG)	246.3	2.98	0.403 ^e	0.101 ^e	3.01	4.23
Monolaurin (C12MG)	274.4	4.04	NG ^e	NG ^e	NG ^a	NG ^a
Monomyristin (C14MG)	302.4	5.10	0.357 ^e	0.125 ^e	7.66	6.39
Monopalmitin (C16MG)	330.5	6.17	0.510 ^e	0.109 ^e	0.41	2.43
Triacylglycerols						
Tributyrin (C4TG)	302.4	2.95	0.671 ^e	0.123 ^e	0.60	2.17
Tricaproin (C6TG)	386.5	6.14	0.643 ^e	0.095 ^e	0.79	2.90
Tricaprylin (C8TG)	470.7	9.33	0.883 ^e	0.110 ^e	0.57	2.38
Tricaprin (C10TG)	554.8	12.52	0.883 ^e	0.124 ^e	0.58	2.23

The experiments were conducted in triplicate and the results were expressed as the averages of three separate experiments that were reproducible within $\pm 10\%$. ^a acetone as a solvent. ^e ethanol as a solvent. ^w water as a solvent. * Lag time was defined as a time until OD₆₆₀ reached 0.15.

MW, molecular weight.

NG, no growth within 240 h.

with the bacterial cell membrane on the growth of *Sporosarcina* sp. S92h, which was previously isolated from surimi, at cold and moderate temperatures. The tested compounds included alkyl gallates, fatty acids, monoacylglycerols, and triacylglycerols, which are mainly used as food additives globally (Table 1). Alkyl gallates have been demonstrated to exhibit antibacterial activities against *Salmonella choleraesuis* (Kubo et al., 2002a), methicillin-resistant *Staphylococcus aureus* (Kubo et al., 2002b), and *Bacillus subtilis* (Kubo et al., 2004). In addition, the antibacterial activities of free fatty acids have been described (Desbois and Smith, 2010). The antibacterial effects of monoacylglycerols against several gram-positive strains belonging to the genera *Staphylococcus*, *Corynebacterium*, *Bacillus*, *Listeria*, and *Streptococcus* were previously reported (Batovska et al., 2009). However, these studies were performed only at moderate temperatures.

To identify the required properties of preservatives against *Sporosarcina* species under cold conditions, the tested compounds were further categorized on the basis of their activities and physicochemical characteristics such as molecular weight and hydrophobic parameters.

2. Materials and methods

2.1. Bacterial strains and chemical reagents

Sporosarcina sp. S92h is a strain isolated from surimi that was deposited at NITE Biological Resource Center under accession number NBRC 111462. Alkyl gallates, free fatty acids, and acylglycerols were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

2.2. Bacterial growth conditions and estimation of specific growth rates

Methyl (MG), ethyl (EG), propyl (PG), butyl (BG), and isoamyl gallates (IG) were dissolved in water at a concentration of 20 mM. Octyl (OG), lauryl (LG), and stearyl gallates (SG) were dissolved in acetone at a concentration of 20 mM. A 50- μ l aliquot of the alkyl gallate solution was added to 4.95 ml of tryptic soy broth (TSB; Becton, Dickinson and Company, Le Pont De Claix, France) medium. Free fatty acids, monoacylglycerols, and triacylglycerols were dissolved in ethanol at a concentration of 40 mM. A 25- μ l aliquot of the fatty acid solution was added to 4.975 ml of TSB medium. Both acetone and ethanol had negligible effects on bacterial growth under all examined conditions (Table 1, Fig. S1). As a primary culture, *Sporosarcina* sp. S92h was cultured in 5 ml of TSB medium at 30 °C for 18 h with reciprocal shaking at 90 rpm. A 5- μ l aliquot of the primary culture was inoculated into 5 ml of TSB medium. The inoculated medium was incubated with seesaw shaking (20 rpm) at 30 °C. The optical density of the culture at 660 nm (OD₆₆₀) was recorded automatically every 15 min for 10 days using a Bio-photorecorder (TN-1506, Advantec Toyo Co. Ltd, Tokyo, Japan). The specific growth rate μ (h⁻¹) was determined via regression analysis during the exponential growth phase according to the following formula:

$$\mu = \Delta \ln OD_{660} / \Delta t,$$

where t is time. The maximum value for the estimated μ was represented as μ_{\max} , which was estimated from the bacterial growth curve (Table 1).

2.3. Calculation of ACD/LogP values

The logarithm of the n -octanol/water partition coefficient (logP) is a widely used hydrophobic parameter for organic compounds. ACD/LogP is the predicted logP using a structure-fragment approach (Osterberg and Norinder, 2001). The ACD/LogP values of the tested compounds were calculated using ACD/ChemSketch 14.01 (Advanced Chemistry Development, Inc., Toronto, Canada).

3. Results and discussion

3.1. Effects of alkyl gallates on growth of *Sporosarcina* sp. S92h

Figs. 1 and 2 show the changes in the OD₆₆₀ of the culture induced by water-insoluble and water-soluble alkyl gallates, respectively. OG and LG completely inhibited the growth of *Sporosarcina* sp. S92h for 10 days at both 10 and 30 °C (Fig. 1, panels A and B), whereas IG exerted a similar growth-inhibitory effect only at 10 °C (Fig. 2, panels A and B). PG and BG moderately decreased the observed OD₆₆₀ values, particularly at 10 °C (Fig. 2, panels A and B). Indeed, the μ_{\max} values for PG and BG at 10 °C were decreased from 0.140 h⁻¹ (the value of the blank at 10 °C) to 0.106 and 0.096 h⁻¹, respectively (Table 1). IG, OG, and LG, which stronger growth-inhibitory effects at 10 °C, have 5, 8, and 12 carbon atoms in their alkyl groups, respectively, whereas the other tested alkyl gallates have less than 5 or more than 12 carbon atoms. The results suggested that the possible existence of an optimal number of carbon atoms between 5 and 12 for growth-inhibitory effect under cold conditions. OG is approved as a food additive with the reference number INS 311 and is used as an antioxidant in foods such as oils,

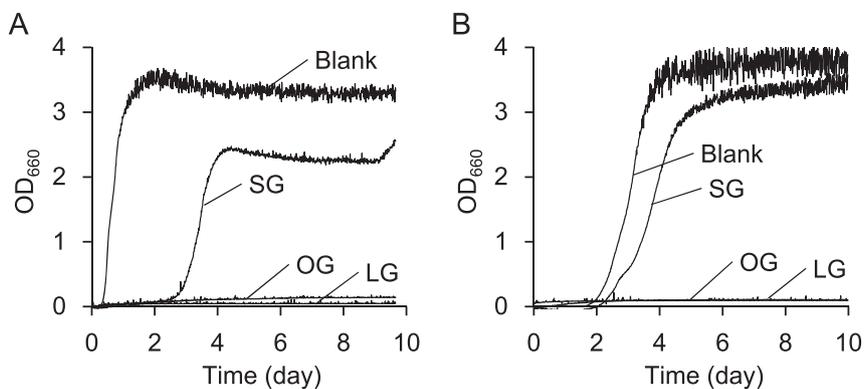


Fig. 1. The changes of the optical density at 600 nm (OD_{660}) of *Sporosarcina* sp. S92h cultures induced by 0.2 mM alkyl gallates (A) at 30 °C and (B) 10 °C using acetone as a solvent. OG, octyl gallate; LG, lauryl gallate; SG, stearyl gallate.

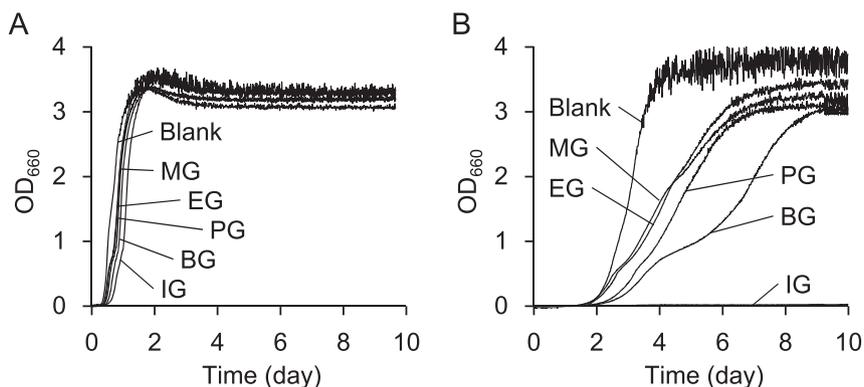


Fig. 2. The changes of the optical density at 600 nm (OD_{660}) of *Sporosarcina* sp. S92h cultures induced by 0.2 mM alkyl gallates (A) at 30 °C and (B) 10 °C using water as a solvent. MG, methyl gallate; EG, ethyl gallate; PG, propyl gallate; BG, butyl gallate; IG, isoamyl gallate.

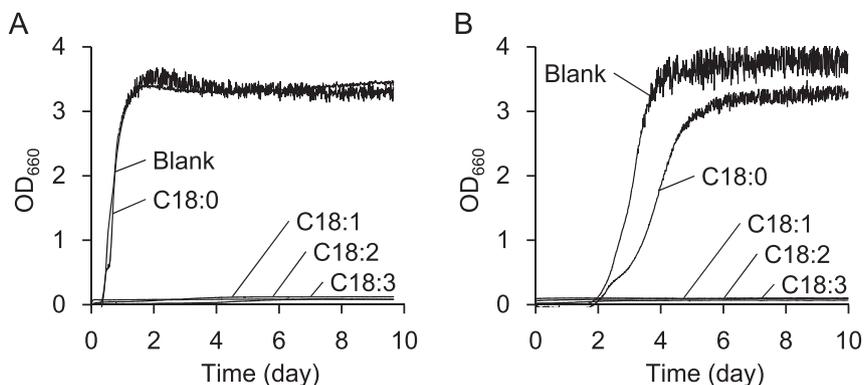


Fig. 3. The changes of the optical density at 600 nm (OD_{660}) of *Sporosarcina* sp. S92h cultures induced by 0.2 mM free fatty acids (A) at 30 °C and (B) 10 °C using ethanol as a solvent. C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, α -linolenic acid.

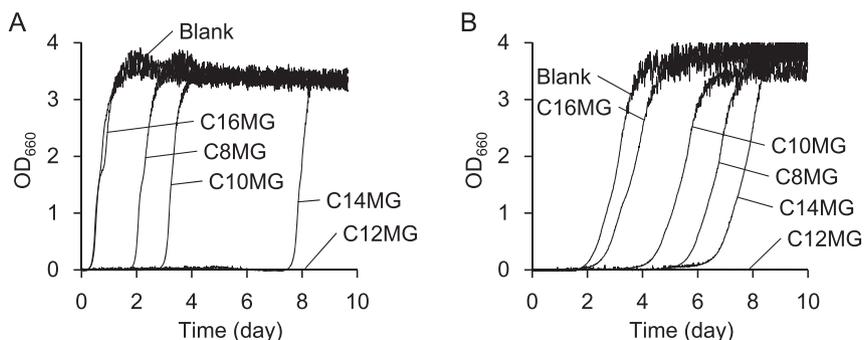


Fig. 4. The changes of the optical density at 600 nm (OD_{660}) of *Sporosarcina* sp. S92h culture induced by 0.2 mM monoacylglycerols (A) at 30 °C and (B) 10 °C using ethanol as a solvent. C8MG, monocaprylin; C10MG, monocaprin; C12MG, monolaurin; C14MG, monomyristin; C16MG, monopalmitin.

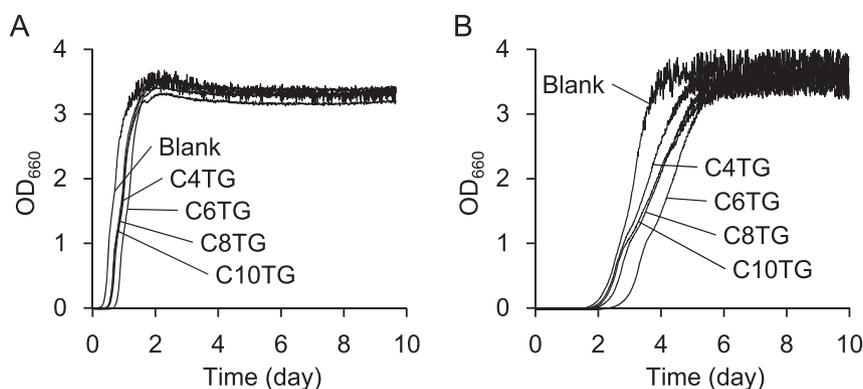


Fig. 5. The changes of the optical density at 600 nm (OD_{660}) of *Sporosarcina* sp. S92h culture induced by 0.2 mM triacylglycerols (A) at 30 °C and (B) 10 °C using ethanol as a solvent. C4TG, tributyrin; C6TG, tricaproin; C8TG, tricapyrin; C10TG, tricaprinn.

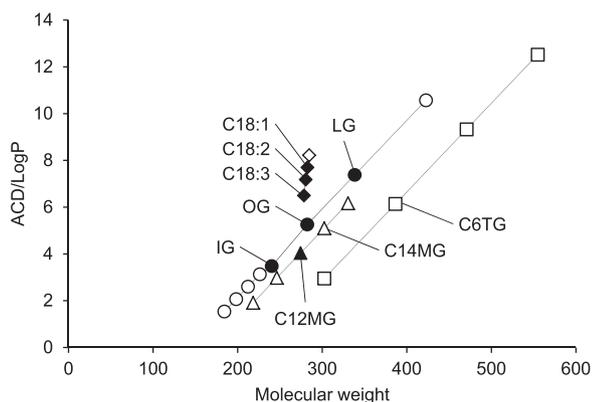


Fig. 6. Relationship between the physicochemical properties of the tested compounds and their growth-inhibitory effects against *Sporosarcina* sp. S92h. The molecular weights and ACD/LogP values of alkyl gallates (circles), free fatty acids (diamonds), monoacylglycerols (triangles), and triacylglycerols (squares) are indicated. The tested compounds with and without growth-inhibitory effects at a concentration of 0.2 mM at 10 °C are indicated by closed and open symbols, respectively. IG, isoamyl gallate; OG, octyl gallate; LG, lauryl gallate; C18:1, oleic acid; C18:2, linoleic acid; C18:3, α -linolenic acid; C12MG, monolaurin; C14MG, monomyristin; C6TG, tricaproin.

margarine, lard, and salad dressings in some countries globally, but not in Japan.

SG affected the observed OD_{660} at 30 °C but not at 10 °C (Fig. 2, panels A and B). In support of this result, μ_{max} at 30 °C for SG was decreased from 0.759 (the value obtained with 1.0% acetone at 30 °C) to 0.127 h⁻¹, whereas that at 10 °C was decreased from 0.140 (the value obtained with 1.0% acetone at 10 °C) to 0.131 h⁻¹ (Table 1). These results suggest that the growth-inhibitory effects of alkyl gallates depend on temperature, and each compound has its own optimal temperature.

3.2. Effects of free fatty acids on the growth of *Sporosarcina* sp. S92h

Fig. 3 shows the changes in OD_{660} induced by stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and α -linolenic acid (C18:3). C18:1, C18:2, and C18:3 completely inhibited the growth of *Sporosarcina* sp. S92h for 10 days at both temperatures (Fig. 3, panels A and B), in line with previous findings for free fatty acids (Desbois and Smith, 2010), whereas C18:0 exerted few growth-inhibitory effects.

3.3. Effects of monoacylglycerols and triacylglycerols on the growth of *Sporosarcina* sp. S92h

Figs. 4 and 5 show the changes in OD_{660} induced by

monoacylglycerols (monocaprylin [C8MG], monolaurin [C10MG], monolaurin [C12MG], monomyristin [C14MG], and monopalmitin [C16MG]) and triacylglycerols (tributyrin [C4TG], tricaproin [C6TG], tricapyrin [C8TG], and tricaprinn [C10TG]), respectively. C12MG completely inhibited the growth of *Sporosarcina* sp. S92h for 10 days at both 10 and 30 °C (Fig. 4, panels A and B). C8MG, C10MG, and C14MG also exerted growth-inhibitory effects at both temperatures. C16MG, which has the longest alkyl chain among the tested monoacylglycerols, exerted few effects on bacterial growth. The results indicated that the optimal number of carbon atoms for growth-inhibitory effect under cold conditions near 12.

Although triacylglycerols exerted few growth-inhibitory effects, C6TG exerted a relatively strong growth-inhibitory effect among the tested triacylglycerols. Indeed, μ_{max} at 10 °C for C6TG was decreased from 0.137 (the value obtained with 0.5% ethanol at 10 °C) to 0.095 h⁻¹ (Table 1).

3.4. Relationship between the physicochemical properties of the tested compounds and their growth-inhibitory effects on *Sporosarcina* sp. S92h under cold conditions

The growth-inhibitory effects of compounds on *Sporosarcina* sp. S92h under cold conditions are summarized in Fig. 6 from the viewpoint of their physicochemical characteristics, i.e., molecular size and hydrophobicity. The molecular weights and ACD/LogP values of the tested compounds are shown in Table 1 and plotted in Fig. 6. We focused on the compounds that completely inhibited the growth of *Sporosarcina* sp. S92h for 10 days at 10 °C at a concentration of 0.2 mM. These compounds, namely IG, OG, LG, C18:1, C18:2, C18:3, and C12MG, have similar molecular weights (range, 240.3–338.4) and ACD/LogP values (range, 3.48–7.70). Other compounds with similar values, such as C14MG and C6TG, also displayed considerable growth-inhibitory effects.

Although further studies are necessary to generalize these findings to other compounds, these data will be useful for exploring more effective compounds. We hope these findings will contribute to controlling the growth of *Sporosarcina* species and maintaining the quality of surimi-based food products.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bcab.2018.11.031.

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