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High Resistance to Oxygen Radicals and Heat Is Caused by a Galactoglycerolipid in *Microbacterium* sp. M874

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***Microbacterium* sp. M874 produced a glyceroglycolipid, di-*O*-12-methyl-tetradecanoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol, at about the 50 μM level. Though the strain was highly resistant to *tertiary*-butyl hydroperoxide (*t*BHP) in a glycolipid-productive medium, the resistance was reduced in a nonproductive medium. Exogenous addition of the glycolipid to the nonproductive culture restored the resistance. This addition also increased the resistance to heat, ethanol, and 4-chloro-1-naphthol, in which oxygen radicals might participate. The parallel relationship found in strain M874 mutants between glycolipid productivity and resistance to *t*BHP or heat suggested that the resistance was mainly caused by the glycolipid. On addition of the glycolipid to a glycolipid-nonproductive culture, it was immediately incorporated into the cells and functioned as an antioxygen radical reagent. Thereafter, its intracellular level remained largely unchanged for at least 5 h, even in the presence of *t*BHP, and its activity was maintained. The glycolipid at 142 μM was sufficient to prevent the cytotoxicity induced by 88.9 mM *t*BHP. The glycolipid production was not induced by pretreatment with a low level of *t*BHP or a sublethal heat shock. In brief, the glycolipid might play an essential role in the prevention of damage by oxygen radicals in the glycolipid-producing bacterium.**

Key words: antioxygen, butyl hydroperoxide, glyceroglycolipid, glycoglycerolipid, stress tolerance.

Cells are often exposed to hazardous levels of oxygen radicals. Oxidative stress is a disturbance in the balance between oxidants and antioxidants. Any pathological situation that increases the oxidative stress or injures the antioxidant defense leads to progressive membrane damage through the oxidation of lipids and proteins. The oxidation process affects membrane integrity, causing altered cell permeability and leakage of the intracellular components (*1*). During the normal course of events, cells and tissues have adequate antioxidant defense systems for membranes, both intracellular and extracellular, of which the latter activity was reported to be appreciably lower (*2*). Moreover, as oxygen radicals are very reactive, a high concentration of scavenger materials in a limited cellular location is necessary for scavenging such radicals (*3*). Therefore, it is probable that an additional reinforcing system of the outerface barriers might exist. When fatty-acid chains become shortened or gain charges through peroxidation, their ability to rotate within the membrane increases and the membrane becomes more fluid (*3*). Therefore, the constituents of a membrane are important for radical-resistance. Some glycolipids have been studied with respect to their membrane-protecting activity against oxygen radicals. Varani *et al.* (*4*) reported that a glycosphingolipid, G_{M1}, and asialo G_{M1} have scavenging activity toward peroxide produced by $H_2O_2/FeSO_4$ treatment. Glycosphingolipids protect against membrane-disordering effects introduced by oxygen radicals (*5–7*). Recently, Matsufuji *et al.* found a cell-bound glycoglycerolipid M874, di-*O*-12-methyl-tetradecanoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol, which had protecting activity against *t*BHP-induced cytotoxicity (*8*).

This report describes the role of the glycolipid in the resistance of the producer strain, *Microbacterium* sp. M874, to oxygen radicals. The strain was clearly resistant to *t*BHP, and some strains of *Microbacterium* have already been reported to be highly heat-resistant (*9*). Heat- and ethanol-stresses have mainly been discussed on the level of oxygen radicals (*3*, *10*). As stress protection is inducible in some cases (*10*, *11*), the production and metabolism of the glycolipid were also examined in this report.

MATERIALS AND METHODS

Microorganisms—Microbacterium sp. M874 was isolated from a soil sample in Japan. Bacteria of other genera were also used as control strains.

*Materials—t*BHP, plumbagin, and menadione sodium bisulfite were purchased, respectively, from MERCK-Schuchardt, Wako Pure Chemical Industries, and the Sigma Chemical Company.

Production and Preparation of M874 Glycolipid—Strain M874 was cultivated in a 250-ml Erlenmeyer flask containing 30 ml of YMPG medium [0.3% yeast extract (Difco), 0.3% malt extract (Difco), 0.5% polypepton (Nihon Pharmaceutical), and 1% glucose, pH 7.0] for 20 h at 28°C. An aliquot (0.3 ml) was added to a 250-ml Erlenmeyer flask containing 30 ml of YSG medium [0.3% yeast extract (Difco), 0.3% soy flour, 2% glucose, 0.05% K_2HPO_4, 0.05%

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Abbreviations. *t*BHP, *tertiary*-butyl hydroperoxyside; MIC, minimum inhibitory concentration.

$MgSO_4 \cdot 7H_2O$, 0.2% NaCl, and 0.2% $CaCO_3$, at pH 7.0] unless otherwise noted. Cultivation was carried out for 3 days at 28°C. The preparation and analysis methods were previously described (*8*).

Radical-Resistance Assay—For the assay in glycolipid-production medium, strains were cultivated in 250-ml Erlenmeyer flasks containing 30 ml of YSG medium, which also contained twofold diluted concentrations of filter-sterilized solution of a radical-producing compound (*t*BHP, plumbagin, or menadione sodium bisulfite), and growth was checked visually. For the assay in glycolipid-nonproductive medium, strains were cultivated in NYE medium (0.4% NH_4NO_3, 0.01% yeast extract, 2% ethanol, 0.1% KH_2PO_4, 0.2% NaCl, and 0.05% $MgSO_4 \cdot 7H_2O$, at pH 7.0). Strains were also cultivated in YSG medium or NYE medium to which 300 μl of methanolic solutions of the M874 glycolipid at various concentrations were initially added.

Heat-Resistance Assay—For the assay in a glycolipid-production medium, strains were cultivated overnight in YSG medium at 28°C with shaking, then incubated at 63°C for 30 min. The culture broth was diluted tenfold with fresh YSG medium several times, and the colony-forming number was calculated by spreading it on YMPG plates and incubating overnight. Control experiments without heat treatment were also done. Strains were also cultivated in NYE medium which initially contained 71 mg/liter (100 μM) of M874 glycolipid, and the heat-resistance was measured.

Extraction and Analysis of Lipids—Lipids were extracted with 10 ml of chloroform/methanol (1:1, v/v) from freeze-dried cells (100 mg). The extract was evaporated to dryness, and the residue was dissolved in 5 ml of chloroform/methanol (1:1, v/v). To remove non-lipid materials, the lipid solution was mixed with 1 ml of 0.88% KCl. The mixture was separated into two phases by centrifugation at 2,200 ×*g* for 5 min. The lower phase was evaporated to dryness, and the amount of phospholipid was determined by assaying the phosphorus content of the extract by the method of Chen *et al.* (*12*). Values of the phosphorus content were multiplied by 25 to give the total amount of phospholipids (*13*).

UV Mutagenesis—Strain M874 was cultivated in YMPG medium, and cells were collected by centrifugation. After washing with a phosphate buffer (1/100 M KH_2PO_4-K_2HPO_4, at pH 7.0), the cell suspension was UV irradiated (20 W, 30 cm distance) for 30 s with shaking. In this case, the viable cell number decreased to the 1% level. The cells were spread on YMPG agar plates containing various concentrations of *t*BHP and incubated for 4 days. *t*BHP-resistant colonies were thus obtained.

Resistance Assay to Ethanol and Toxic Organic Substances—Strain M874 was cultivated overnight in a YMPG medium. An aliquot (30 μl) was added to a 20-ml test tube containing 3 ml of NYE medium and incubated for 4 h at 28°C with shaking. Then 30 μl of methanolic solution of the glycolipid was added to give a final concentration of 100 mg/liter, and the culture was incubated at 28°C for 2 h. For the ethanol resistance assay, ethanol was added at various concentrations. After incubation for 40 h with shaking, the colony-forming number was measured. Control experiments without the glycolipid were also done. For the resistance assay to 4-chloro-1-naphthol, 30 μl of a 100-fold concentration of the methanolic solution was added

Test of Glycolipid Incorporation and Metabolism—Strain M874 was cultivated in YMPG medium. An aliquot (0.3 ml) was added to a 250-ml Erlenmeyer flask containing 30 ml of NYE medium and incubated at 28°C for 15 h. Then the glycolipid was added at a final concentration of 100 mg/liter, and incubation was continued. Samples of 1 ml were periodically withdrawn, cells were collected by centrifugation, and the supernatant and cell debris were separated. The supernatant was mixed with an equal volume of solvent (chloroform:methanol = 1:1). After twice washing the cell debris with 1 ml of fresh NYE medium, a 1-ml cell suspension was mixed with 1 ml of the solvent. The glycolipids in the broth supernatant and in the cell debris were measured by a TLC method (*8*). For analysis of the glycolipid incorporation and metabolism in the presence of *t*BHP, various concentrations of *t*BHP were added to a 15-h culture of strain M874 in NYE medium. Ten minutes later, 100 mg/liter of the M874 glycolipid was added to the culture, and the intracellular and extracellular glycolipids were measured.

Colony-Forming Tests with the Addition of tBHP and/or the M874 Glycolipid—Strain M874 was cultivated in YMPG medium. An aliquot (30 μl) was added to a 20-ml test tube containing 3 ml of NYE medium and incubated at 28°C with shaking. Various concentrations of the glycolipid were added to the culture at 2.5 h, and various concentrations of *t*BHP were added at 3 h unless otherwise described. The colony-forming number was measured periodically thereafter. The M874 glycolipid and *t*BHP were added as 1/100 volume of 100-fold concentrations of methanolic and aqueous solution, respectively. Blank conditions were accomplished by the addition of methanol and water. $FeSO_4 \cdot 7H_2O$ solution was similarly added after the filter sterilization.

RESULTS

Relationship of Glycolipid Concentration and Radical-Resistance of Microbacterium sp. M874—The C- and N-sources were tested for glycolipid production of strain M874. In NYE medium, no detectable amount of the glycolipid was produced, while in YSG medium, the strain produced the glycolipid proportionally with the growth (Fig. 1).

The strain was more sensitive to *t*BHP than the other oxygen radical-producing compounds, plumbagin and

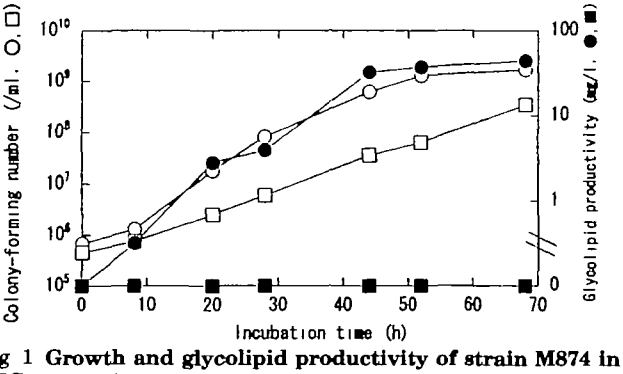

Fig 1 **Growth and glycolipid productivity of strain M874 in YSG and NYE media.** Strain M874 was cultivated in YSG medium (○, ●) and NYE medium (□, ■) and periodically sampled Growth was monitored by colony-forming number on a YMPG plate. Glycolipid was monitored by a TLC method

menadione (Table I). The resistance to *t*BHP was compared in three media: YSG, NYE, and NYE plus 100 mg/liter (142 μM) of the M874 glycolipid. The MICs of *t*BHP in these media were 250, 31, and 500 mg/liter, respectively. In the tests for plumbagin and menadione, the resistance level in NYE medium was less than in YSG medium and was increased by the addition of the M874 glycolipid.

When the M874 glycolipid was added to an NYE culture, the resistance to *t*BHP increased proportionally to the added glycolipid concentration only at more than 10 mg/liter of the glycolipid (Fig. 2).

As a control, the phosphoglycerolipid productivity in each culture was measured. The level was similar in each case: 4.1 mg per g dry cell weight (YSG), 3.7 (NYE), and 3.5 (YSG plus 100 mg/liter of the glycolipid).

Comparison of Microbacterium sp. M874 with Bacteria of Other Genera—The *t*BHP-resistance of strain M874 was compared with those of five representative bacteria of different genera. In YSG medium, the resistance level of strain M874 to *t*BHP (125 mg/liter) was higher than those of all the other bacteria (31 mg/liter) (Table II). The resistance level of all the other five bacteria in NYE medium was the same as that in YSG medium (MIC; 31 mg/liter). When the M874 glycolipid was exogenously added to the NYE medium, all the bacteria showed increased *t*BHP-resistance, though the degrees of increase were different.

The heat resistance of strain M874 was compared with those of the five other bacteria in YSG medium by measuring the colony-forming ability after the heat treatment of each culture broth. In strain M874, the rate of viable cells was remarkably higher than in the other bacteria, which indicated that strain M874 was significantly heat-resistant (Table II). The other five bacteria did not produce any detectable level of glycolipids with radical-protecting ability.

Comparison of Microbacterium sp. M874 with Its Mutant Strains—Strain M874 was UV-mutagenized, and *t*BHP-resistant mutants were obtained. The mutant strains were measured for glycolipid productivity and resistance to *t*BHP in YSG medium. The resistance to *t*BHP of each strain was comparable to the glycolipid productivity (Fig. 3A). The parent strain produced 40 mg/liter of the M874 glycolipid and a highly *t*BHP-resistant mutant, No. 2550, produced 110 mg/liter of the glycolipid. These results indicated that the M874 glycolipid played an important role in the radical-protection of *Microbacterium* sp. M874. The glycolipid accounted for 0.4% of the dry cell weight in strain M874 and 1.1% in the mutant No. 2550. Heat-resistance in YSG medium also increased in proportion to the glycolipid productivity (Fig. 3B).

TABLE I **Sensitivity to oxygen radical-producing compounds.**

Medium	Minimum inhibitory concentration (mg/liter) of		
	*t*BHP	Plumbagin	Menadione
YSG	250	1,000	1,000
NYE	31	250	250
NYE + glycolipid	500	>2,000	>2,000

In "NYE + glycolipid," M874 glycolipid was initially added at 100 mg/liter "Menadione" means menadione sodium bisulfite

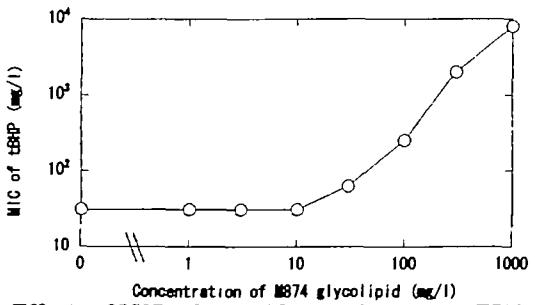

Fig. 2. **Effects of M874 glycolipid on resistance to *t*BHP.** M874 glycolipid was initially added at various concentrations to NYE medium and strain M874 was cultivated. Resistance to *t*BHP was monitored by checking growth visually

TABLE II ***t*BHP and heat resistance of various bacteria.**

	Minimum inhibitory concentration (mg/liter) of *t*BHP in			Cell viability after heat treatment
	YSG	NYE	NYE+ glycolipid	
Microbacterium sp M874	250	31	500	1.25×10^{-2}
Bacillus subtilis M15	31	31	125	7.42×10^{-7}
Escherichia coli H101	31	31	125	7.82×10^{-6}
Klebsiella pneumoniae H12	31	31	125	9.86×10^{-7}
Pseudomonas fluorescens S272	31	31	63	7.42×10^{-7}
Rhodococcus equi S50	31	31	250	4.56×10^{-4}

MIC was measured in YSG and NYE media, and NYE medium containing 100 mg/liter of M874 glycolipid Rate of living cells was measured after treatment of YSG culture at 63°C for 30 min as described in "MATERIALS AND METHODS."

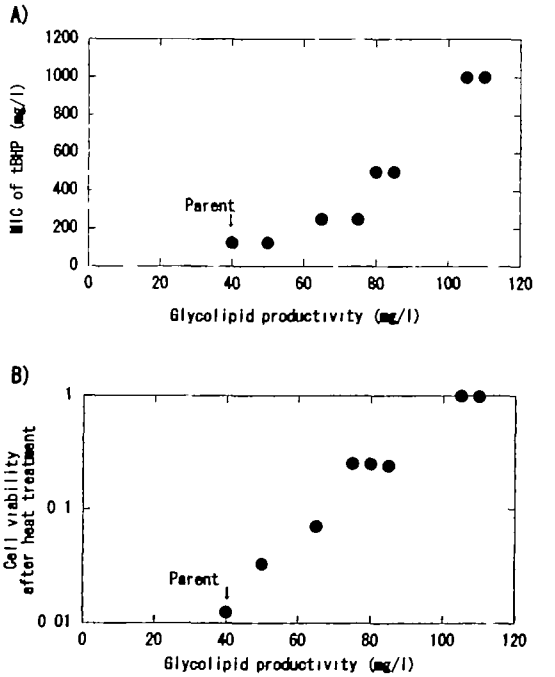

Fig. 3. **Comparison of strain M874 with its mutant strains in resistance to *t*BHP (A) and heat (B).** Strains were cultivated in YSG medium for 3 days and glycolipid productivity was measured. MIC of *t*BHP was measured by cultivating the strains in YSG medium containing various concentrations of *t*BHP Resistance to *t*BHP was monitored by checking growth visually. Cell viability after treatment at 63°C for 30 min is expressed as the colony-forming number relative to untreated cells.

The growth rates of the parent strain and strain No. 2550 were almost identical (data not shown). As a control, the amounts of phosphoglycerolipid were also similar: 4.1 mg per g dry cell weight (parent) and 4.4 mg/g (No. 2550).

Miscellaneous Functions of Exogenous Glycolipid in NYE Medium—Heat resistance of strain M874 was tested in NYE medium in the presence of various concentrations of the glycolipid. The results (Fig. 4A) indicated almost complete heat resistance at 63°C when 300 mg/liter of the glycolipid was added.

Ethanol tolerance of strain M874 was tested in NYE medium containing 100 mg/liter of the glycolipid (Fig. 4B). The glycolipid increased the growth level (OD_{550}) in media containing 4–12% of ethanol. Similarly, the glycolipid increased the MIC-value of a toxic organic substance, 4-chloro-1-naphthol (Fig. 4C), as well as 3-indole acrylate and diphenylamine (data not shown).

Test of Glycolipid Incorporation and Metabolism—The cellular incorporation rate of the exogenously added glycolipid was measured. When 100 mg/liter of the glycolipid was added to an ENA culture of strain M874, 78% was incorporated into the cell within 5 min, and the intracellular level was totally maintained for at least 5 h (Fig. 5A).

The effect of *t*BHP on the glycolipid incorporation and metabolism was next studied (Fig. 5B). At all concentrations tested, *t*BHP did not affect the glycolipid incorporation rate or the total glycolipid concentration (intracellular plus extracellular), suggesting that *t*BHP did not increase the metabolism rate of the M874 glycolipid.

Effects of Glycolipid and tBHP on Colony Forming Number—The effect of the M874 glycolipid was further studied by using the culture of strain M874 in a glycolipid-nonproductive medium, NYE. The glycolipid at a concentration of more than 100 mg/liter was growth-promoting (Fig. 6A), but lower concentrations did not affect the growth rate. Without the glycolipid, the colony-forming ability decreased markedly when more than 500 mg/liter of *t*BHP was added to an NYE culture; but with the glycolipid, the colony-forming ability gradually increased (Fig. 6B).

When 100 mg/liter of the M874 glycolipid was added to an NYE culture of strain M874 from 3 h to 1 min before the addition of 500 mg/liter of *t*BHP, the colony-forming num-

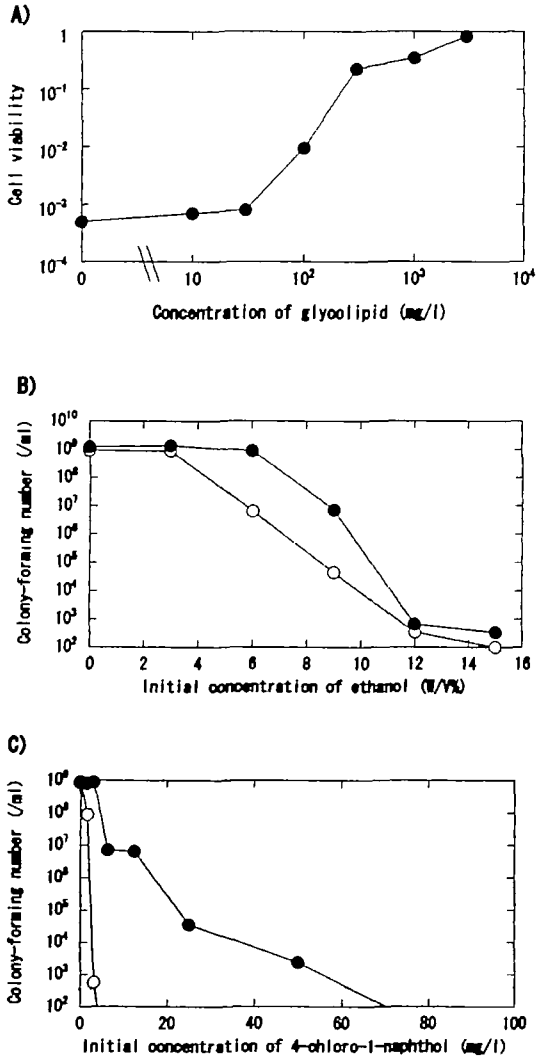

Fig. 4. **Miscellaneous functions of exogenous glycolipid in NYE medium culture.** A) Heat-resistance assay Cell viability in NYE culture containing various concentrations of M874 glycolipid was measured after treatment at 63°C for 30 min and expressed relative to that in an untreated culture. B) Ethanol-resistance assay Colony-forming number in NYE medium culture containing various initial concentrations of ethanol. Symbols. ●, with 100 mg/liter M874 glycolipid; ○, without the glycolipid. C) Resistance assay to 4-chloro-1-naphthol. Colony-forming number in NYE medium culture containing various initial concentrations of the compound Symbols: ●, with 100 mg/liter M874 glycolipid; ○, without the glycolipid.

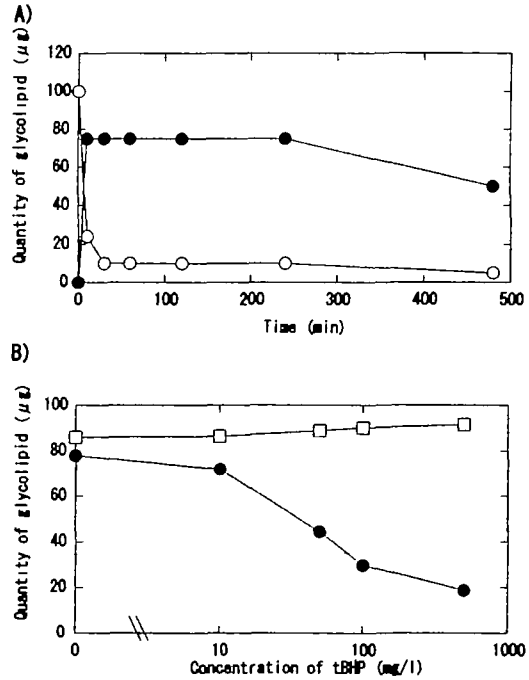

Fig. 5. **Rate of cellular incorporation and metabolism of exogenouly added glycolipid.** A) M874 glycolipid (100 μg) was added to test tubes containing 1 ml of NYE culture, and the quantity of intracellular (●) and extracellular (○) glycolipid was periodically measured by HPLC B) M874 glycolipid (100 μg) and various concentrations of *t*BHP were added to test tubes containing 1 ml of NYE culture, and the quantity of intracellular (●) and total (intracellular plus extracellular; □) glycolipid were measured 10 min later

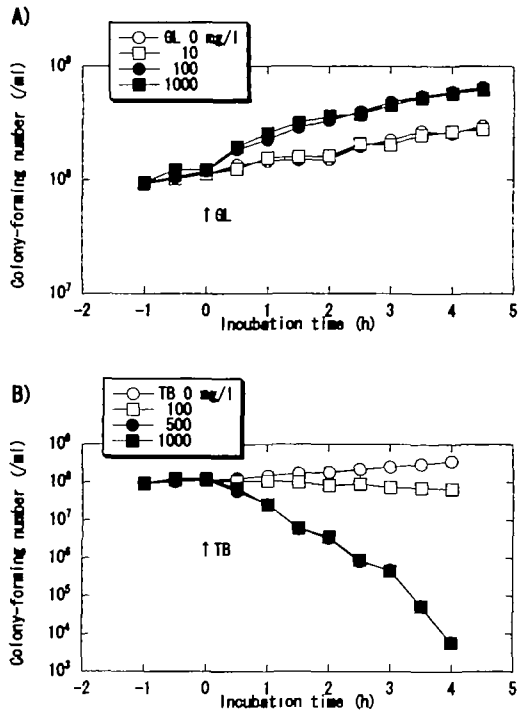

Fig. 6. **Effects of glycolipid (A) and *t*BHP (B) on colony-forming number.** Details are described in "MATERIALS AND METHODS" Strain M874 was cultivated, and the time of addition of glycolipid or *t*BHP was defined as incubation time 0. GL and TB represent M874 glycolipid and *t*BHP, respectively.

ber was double the blank level (namely, without addition of glycolipid and *t*BHP) (Fig. 7A). When the M874 glycolipid was added to the culture after the addition of *t*BHP, the decrease in the colony-forming number stopped immediately and the number started to increase at a rate higher than in the absence of the glycolipid.

To evaluate the roles of protein synthesis and energy consumption in the cellular protection by the glycolipid, a subcidal level of chloramphenicol (5 mg/liter) or sodium azide (15 mg/liter) was added 30 min before the addition of the glycolipid, and colony-forming number was periodically measured after *t*BHP addition (Fig. 7B). Neither chloramphenicol nor sodium azide influenced the function of the glycolipid, suggesting that protein synthesis and energy consumption might not be important.

Lipid peroxidation is one of the main forms of cellular injury caused by oxygen radicals, and the ferrous ion was reported to greatly accelerate cellular injury through the lipid hydroperoxides (*14*). To ascertain the cause of the cytotoxicity of *t*BHP, the effect of Fe^{2+} was tested. The cytotoxicity of *t*BHP to strain M874 was increased by the addition of $FeSO_4 \cdot 7H_2O$, and the exogenous M874 glycolipid (100 mg/liter) rescued the strain from the toxicity caused by the coaddition of Fe^{2+} and *t*BHP (Fig. 7C).

Inducibility Test of the Glycolipid Production—Neither a low level of *t*BHP (Fig. 8A) nor sublethal heat-shock treatment (Fig. 8B) increased the glycolipid production, suggesting that the productivity was constitutive.

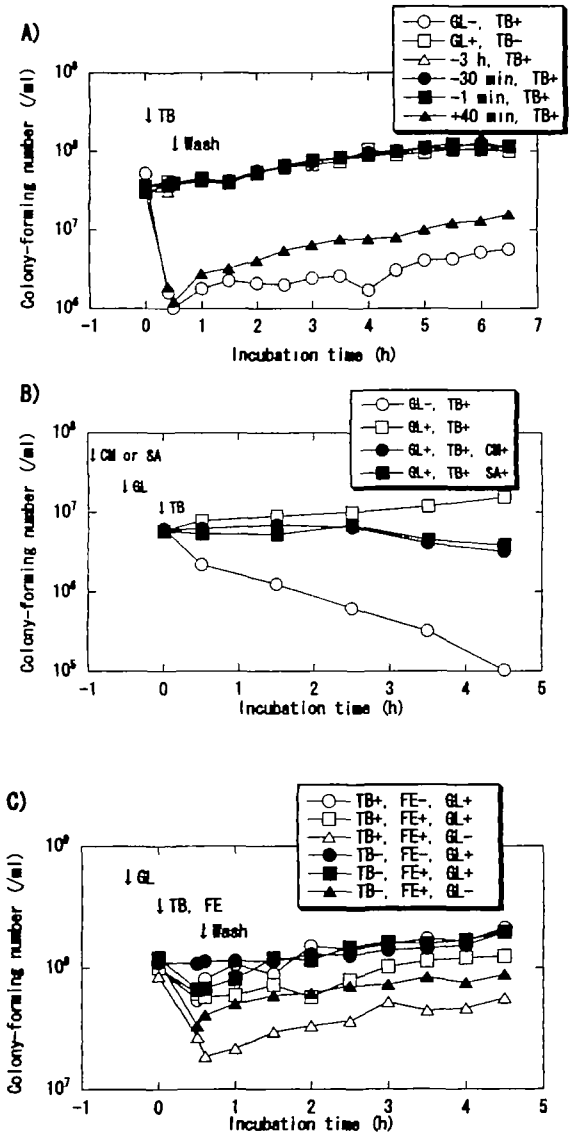

Fig. 7 **A) Effect of addition time of glycolipid on the rescue from *t*BHP-induced cell death.** Strain M874 was cultivated with the addition of 100 mg/liter of glycolipid at different times. The addition time of 500 mg/liter of *t*BHP was defined as incubation time 0. At 0.5 h, cells were washed by centrifugation and resuspended in fresh medium. Cell culture was periodically sampled, and colony-forming number was monitored **B) Effect of chloramphenicol (CM) and sodium azide (SA) on the function of the glycolipid.** Strain M874 was cultivated with the addition of CM or SA (at −1 h), glycolipid (at −0 5 h), and *t*BHP (at 0 h). The concentrations were 5, 15, 100, and 500 mg/liter, respectively. Cell culture was periodically sampled, and colony-forming number was monitored. **C) Effect of glycolipid on the additional cellular lesion induced by *t*BHP and Fe^{2+}.** Strain M874 was cultivated with the addition of glycolipid (GL, at −0.5 h), *t*BHP (TB, at 0 h), $FeSO_4 \cdot 7H_2O$ (FE, at 0 h). The concentrations were 100, 500, and 100 mg/liter, respectively At 0 5 h, cells were washed by centrifugation and resuspended in fresh medium Cell culture was periodically sampled, and colony-forming number was monitored.

DISCUSSION

The cellular protection mechanism against oxygen radicals has been widely studied but not fully clarified. The results

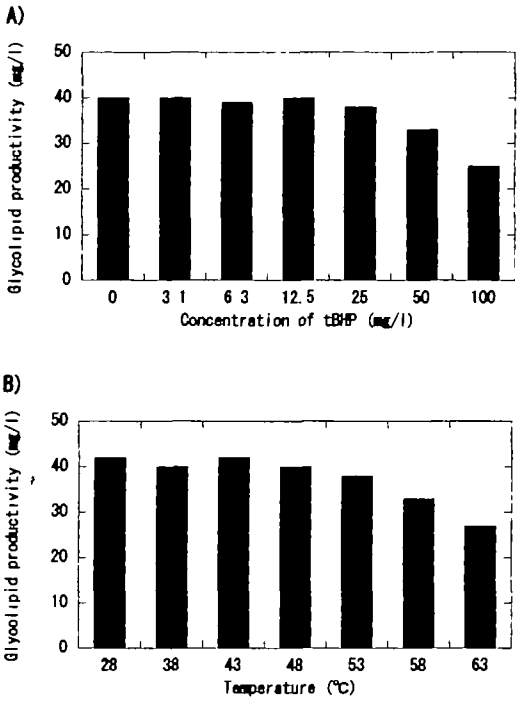

Fig 8. **Inducibility of the glycolipid production by *t*BHP (A) and heat shock (B)** Strain M874 was cultivated in YSG medium for 3 days at 28°C with shaking, and glycolipid productivity was measured by TLC. In (A), various concentrations of *t*BHP were initially added. In (B), heat-shock treatment for 5 min was performed at various temperatures on day 2.

described in this report support the idea that the M874 glycolipid plays an important role in radical-protection of the glycolipid producer, *Microbacterium* sp. M874. (i) Exogenous addition of the glycolipid to a nonproductive culture increased the resistance to *t*BHP (Table II). (ii) The glycolipid producer, strain M874, was more resistant to *t*BHP in a glycolipid-productive medium than bacteria of some other genera (Table II). (iii) More resistant mutants of strain M874 to *t*BHP produced a higher amount of the glycolipid (Fig. 3A). As scavenger substances such as glutathione or L-cysteine decreased the toxicity of *t*BHP to strain M874 (data not shown), *t*BHP might function on the level of oxygen radicals. *t*BHP produces lipid hydroperoxide species and, in the presence of transition metal complexes, especially iron salts, cellular injury through the lipid hydroperoxides is greatly accelerated (*14*). Here, the toxicity of *t*BHP was increased by $FeSO_4$ and totally reversed by the M874 glycolipid (Fig. 7B).

Some glycosphingolipids of eukaryotes have been studied with respect to their membrane-protecting activity against oxygen-radicals (*4–7*), and their role as second messengers for regulating the intracellular signal transduction pathway has been emphasized. In our previous experiment (*8*), G_{M1} and ceramides had no rescuing effect against *t*BHP-induced cellular lesion in some bacteria. But, it is probable that glycoglycerolipids in bacteria play an alternative role to glycosphingolipid in eukaryotes.

TLC, HPLC, and ^{1}H-NMR analyses have shown that the M874 glycolipid is incorporated into the bacterial cell in an intact form and that the M874 glycolipid is not oxidized or modified by the exposure to *t*BHP. The glycolipid was effective only at concentrations greater than 14.2 μM (10 mg/liter), and the glycolipid equivalents at 1.42 mM (1,000 mg/liter) reversed the toxicity of up to 88.9 mM (8,000 mg/liter) of *t*BHP (Fig. 2). These data suggest that the M874 glycolipid might play a different role from radical-scavenger molecules such as ascorbic acid, glutathione, or α-tocopherol. The concentration of the glycolipid (57 μM or more) produced in YSG medium is similar to the concentration of the representative scavenger molecules in human blood (5–25 μM of glutathione, 40–140 μM of ascorbic acid, and 10–40 μM of α-tocopherol) (*15*), although the glycolipid was found only in the cell debris.

An inducible membrane-repair response has been widely proposed. When oxygen radicals disrupt membrane functions at nonlethal doses, cells acquire the ability to rapidly recover from the loss of membrane functions (*16*, *17*). Our data indicated that the production and the metabolism of the glycolipid was not inducible by a subcidal level of *t*BHP or heat shock (Fig. 8). Protein synthesis or energy consumption also appeared to be unnecessary for the glycolipid function, suggesting that the M874 glycolipid might not be an inducer of a radical-scavenging cascade. These constitutive and highly-efficient functions might be important for an indispensable role in the resistance to oxygen radicals.

Microbacterium sp. M874 was significantly resistant to heat, ethanol, and 4-chloro-1-naphthol in a glycolipid-productive medium, compared to some representative bacteria of different genera (partly in Table II). Many strains of *Microbacterium* have already been reported to be heat-resistant (*9*). In this report, the exogenous addition of the glycolipid increased the heat resistance (Fig. 4A), ethanol resistance (Fig. 4B), and resistance to 4-chloro-1-naphthol (Fig. 4C) in a glycolipid-nonproductive medium. These data suggested that resistance to these stresses was exerted through the function of the glycolipid. As the coaddition of *t*BHP and 4-chloro-1-naphthol (or ethanol) caused a synergistic toxicity to the strain (data not shown), 4-chloro-1-naphthol and ethanol might be harmful oxidants. The glycolipid at a concentration greater than 100 mg/liter was growth-promoting (Fig. 6A), which might also be a result of the radical-rescuing activity of the glycolipid. Moreover, it will be important to study the additional effects of the glycolipid on miscellaneous physiological functions, because oxygen-radicals participate in many physiological reactions and the addition of M874 glycolipids to cultures of bacteria of other genera increased their resistance to *t*BHP (Table II).

This paper focuses mainly on the roles of the glycolipid in *Microbacterium* sp. M874. However, Table II clearly indicates that the glycolipid functioned in other bacteria, although the degree of its function varied among strains. The functions of the glycolipid in other organisms is an intriguing theme for future study.

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