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ARTICLE

A comparative study of fatty acid profile and formation of biofilm in *Geobacillus gargensis* exposed to variable abiotic stress

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Abstract: Understanding bacterial fatty acid (FA) profile has a great taxonomic significance as well as clinical importance for diagnosis issues. Both the composition and nature of membrane FAs change under different nutritional, biotic and (or) abiotic stresses, and environmental stress. Bacteria produce both odd-carbon as well as branched-chain fatty acids (BCFAs). This study was designed to examine the effect of abiotic pressure, including salinity, temperature, pH, and oxinic stress on the growth, development, and FA profile in thermophilic *Geobacillus gargensis*. Under these stresses, 3 parametric ratios, 2-methyl fatty acids/3-methyl fatty acids (iso-/anteiso-FAs), BCFAs/straight-chain saturated fatty acids (SCSFA), and SCSFAs/straight-chain unsaturated fatty acids (SCUFA), in addition to total lipids affected by variable stresses were measured. Our results indicate that the ratio of total iso-/anteiso-FAs increased at the acidic pH range of 4.1–5.2 and decreased with increasing pH. The reverse was true for salt stress when iso-/anteiso-FAs ratio increased with salt concentration. The BCFAs/SCSFAs and SCSFAs/SCUFAs ratios increased at neutral and alkaline pH and high salt concentration, reduced incubation time, and comparatively high temperature (55–65 °C) of the growth medium. The bacterial total lipid percentage deceased with increasing salt concentration, incubation period, but it increased with temperature. The formation of extracellular polymeric substances was observed under all stress conditions and with the addition of sodium dodecyl sulfate (2 and 5 mmol/L) to the growth medium. The membrane phospholipid composition of the bacterium was analyzed by thin-layer chromatography.

Key words: Geobacillus gargensis, branched-chain fatty acids, extracellular polymeric substances, biofilm, abiotic stress.

Résumé : La compréhension des profils d'acides gras bactériens revêt une importance capitale pour la taxonomie, sans oublier leur portée clinique et diagnostique. La composition et la nature des acides gras (AG) membranaire varient selon les stress nutritionnels, biotiques et (ou) abiotiques et environnementaux. Les bactéries produisent des acides gras à chaînes impaires de carbone de même que des acides gras à chaîne ramifiée (AGCR). La présente étude a été conçue pour cerner les effets de la pression abiotique, dont la salinité, la température, le pH et le stress oxynique, sur la croissance, le développement et le profil d'acides gras du thermophile *Geobacillus gargensis*. Sous l'effet de ces stress, on a mesuré trois rapports paramétriques, à savoir les acides gras 2-méthylés/acides gras 3-méthylés (AG-iso/-antéiso); les AGCR/acides gras à chaîne droite saturée (AGCDS); et les AGCDS/acides gras à chaîne droite insaturée (AGCDI); auxquels on a ajouté les lipides totaux. Nos résultats indiquent que le rapport d'acides gras totaux iso/antéiso augmente dans l'intervalle de pH de 4,1 à 5,2 pour ensuite baisser à mesure que le pH grimpe. On a observé l'effet inverse en présence de stress salin, du fait que le rapport d'AG-iso/antéiso suivait l'évolution de la concentration de sel. Les rapports AGCR/AGCDS et AGCDS/AGCDI ont subi une hausse à un pH neutre et alcalin et à salinité élevée, lors d'un temps d'incubation raccourci et à des températures du milieu de culture relativement élevées (55–65 °C). Le pourcentage des lipides bactériens totaux a varié à l'inverse de la concentration de sel et du temps d'incubation, mais a augmenté selon la température. On a observé la formation de substances polymériques extracellulaires sous l'effet de toutes les conditions stressantes et conséquente de l'ajout de dodécylsulphate de sodium (2 et 5 mmol/L) dans le milieu de culture. On a analysé la composition en phospholipides membranaires de la bactérie par chromatographie sur couche mince. [Traduit par la Rédaction]

Mots-clés : Geobacillus gargensis, acides gras à chaîne ramifiée, substances polymériques extracellulaires, biofilm, stress abiotique.

Introduction

Microbial communities have a good potential to adapt to a wide variety of environmental stresses, including extreme anoxia, temperature, salt stress, desiccation, pH, ionizing radiation, hydrostatic pressure, nutrient accessibility, and toxic chemicals (Dartnell et al. 2010; Makhdoumi-Kakhki et al. 2012; Quintana et al. 2013). There are several microbes that can survive in more than one type of extreme environmental condition and these are classified as polyextremophiles (Dartnell et al. 2012; Kumar et al. 2012; Stock et al. 2012). The extremophilic communities are physiologically important, since they have potential applications in medicine, including antibiotics, local application in skin care products, stable enzymes in biosensors, and drug excipients (Irwin 2010). Thermophiles, as extremophiles, are a good source of thermostable enzymes and have been exploited for pharmaceutical and many other industrial applications (Lin et al. 2013; Oliveira et al. 2013; Panda et al. 2013; Park et al. 2013). It is known that microbial adaptation is regulated by changes in cellular enzymes, membrane lipids, and their morphology, leading to a variation in functional dynamics and plasticity through redox and signaling systems of the organism (Contera et al. 2010; Pocock et al. 2011; Diakogiannis et al. 2013).

Geobacillus gargensis has been described as a heterotypic synonym of *Geobacillus thermocatenulatus* (Dinsdale et al. 2011). *Geobacillus gargensis* is a spore-forming species isolated from hot springs in northern Transbaikal region of Russia (Nazina et al. 2004). It can

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grow at 60–65 °C on different sugars, hydrocarbons, or carboxylic acids as carbon sources. Its potential for hydrocarbon degradation makes this organism important for environmental remediation for oil-contaminated soil. We have explored the capacity of *Geobacillus kaustophilus* and *G. gargensis* for the biotransformation of progesterone that resulted in a spectrum of steroid-transformed products that could previously not be synthesized through traditional organic synthesis (Al-Khalaf et al. 2013). This highlights the versatility of this organism.

Fatty acids (FAs) have an important biological role in cell membranes. Not only are they responsible for membrane plasticity but they are also clinically important molecules (Wolfram and Adam 1980; Das 1991). For example, an increase in the polyunsaturated FAs increases the fluidity and also increases membrane receptors and their affinity to the respective hormones and (or) growth factors and consequently to cell signaling (Das 2005*a*). In addition, polyunsaturated FAs are prone to oxidative modifications and may have antiviral and antibacterial activities that play an important biological role in self-defense (Das 1985; Giamarellos-Bourboulis et al. 2004). Different amounts and types of FAs are synthesized in response to diverse stresses, and this property has been linked with the self-defense of the organism (Das 2005*b*).

Branched-chain iso- and anteiso-FAs are the major constituents of bacterial membrane lipids. ω-Cyclohexyl and ω-cyclopentyl FAs are also present in some bacterial membrane lipids. Bacterial growth is supported by the branched-chain FAs. Since these FAs are the major cellular components in bacteria, they are used in the identification and classification of bacteria (Suzuki and Komagata 1983; Kaneda 1991). The accumulation or release of FAs is known to depend on external stimuli, such as hormones or nutritional supply (Dirusso and Balck 2004). In the present study, we set out to investigate the effect of variable temperature, incubation time, pH, salt stress, and anoxic stress on the growth pattern of *G. gargensis* and its adaptation to stress by variations in membrane lipids and their constituents. Previously, in *G. gargensis*, pentadecanoic, hexadecanoic, and heptadecanoic acids have been identified as major membrane FAs.

Material and methods

All reagents and solvents were of analytical grade and were obtained from E. Merck (Darmstadt, Germany) and Scharlau (Barcelona, Spain). Before use, the solvents were redistilled under reduced pressure using a Rotavapor (Büchi, Flawil, Switzerland). Inorganic salts and standard phospholipids (PLs), L-α-phosphatidylethanolamine, L-α-phosphatidylglycerol, L-α-phosphatidylcholine, diphosphatidylglycerol, cardiolipin, and L-α-phosphatidic acid, were obtained from Sigma-Aldrich (Munich, Germany). Culture media were purchased from Flucka Riedel-deHaen, (Seelze, Germany) and Scharlau, (Barcelona, Spain). Olympus biological microscopes (models CHS and CHT) and phase-contrast microscope (model CHT) (Olympus Optical Co., Ltd., Tokyo, Japan) were used for microscopy work. Bacterial inoculations and microbial procedures were carried out in a laminar flow hood (Labcaire TC1200, Labcaire Systems Ltd., Portway, UK) under aseptic conditions. A standard bacterial fatty acid methyl ester (FAME) mixture with 24 components containing 6 hydroxy FAMEs and other positional and geometric isomeric FAMEs, CP Mix No. 47080-U, commonly found in bacteria, was purchased from Supelco (Bellefonte, Pennsylvania, USA). Silica Sep-Pak, the classic silica cartridges, was obtained from Waters Corp. (Milford, Massachusetts, USA). Syringe filters, 0.2 μm pore size, were obtained from Pall Corporation (USA). Micro-Mate interchangeable syringes were obtained from Popper & Sons, Inc. (New Hydepark, New York, USA). Gas chromatography – mass spectrometry (GC–MS) data were collected on an Agilent instrument (model 5973, Palo Alto, California, USA) with a network mass selective detector, interfaced with an Agilent gas chromatography (model 6890-A) system and NIST library database.

Organism culture and maintenance

Isolates of *G. gargensis* (type strain DSM 15378) were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, German Collection of Microorganisms and Cell Cultures) as dry lyophilized cells. Isolates were suspended in nutrient broth (Difco, Becton Dickinson and company, Sparks, Maryland, USA), prepared in 10% Castenholz mineral salts with 86 mmol/L NaCl, pH 7 (adjusted with 1 mol/L NaOH or HCl) and incubated at 60 °C in a shaker–incubator (Gallenkamp orbital incubators model INR-250, London, UK) at 120 r/min for 24 h, as described in the literature (Ramaley and Hixson 1970). The broth culture was subcultured on nutrient agar plates under the same conditions and stored at 4 °C until used. Bacterial strain was subcultured every month using standard microbial techniques. The sampling was done every 30 min, and the growth curve was constructed using optical density (OD) vs time. Bacterial cells were stained violet with Gram stain and the violet color was taken as Gram-positive bacilli.

Since peptide–lipid interactions, cell adhesion, extracellular polymeric substances (EPS) formation, and membrane plasticity are all affected by stress, we looked at the bacterial cell membrane morphology, by microscopy, under variable abiotic stresses and also with the addition of the surfactant sodium dodecyl sulfate (SDS) at 2 and 5 mmol/L concentrations to the culture medium under each applied abiotic stress.

Processing of *G. gargensis* for scanning electron microscopy (SEM)

Bacterial cells, cultured under optimum growth conditions using nutrient agar, were exposed to variable abiotic stresses, such as temperature (45–70 °C), incubation time (4.5 h–10 days), pH (4.1–8.1), salinity (14–510 mmol/L NaCl), anoxic or oxic settings. The cells from each stress study were suspended in 3% glutaraldehyde fixative with phosphate buffer and kept on a rotator for 2 h at room temperature, followed by overnight storage at 4 °C in a refrigerator. Samples were rinsed 3 times with 5 mmol/L phosphate buffer (pH 6.5), 10 min each rinse. Cells were collected by centrifugation and suspended in 1% osmium tetroxide for 2 h for postfixation. This was followed by 3 rinses with buffer, 10 min each rinse. Samples were dehydrated using acetone gradient 30%–100% with 10 min intervals. The chemically dehydrated samples were conclusively dehydrated in a critical point dryer. The dried samples were mounted on a stub by using carbon double adhesive tape and the samples were metal coated with gold in the sputter coater. The stubs were stored in a desiccator until observed under SEM.

Extraction of lipids

Freeze-dried bacterial cells (100 mg), obtained from variable stress growth experiments, were suspended in a 1 mL mixture of methanol–chloroform–isopropanol (2:1:0.5, by volume) and probe-sonicated (Biologics Inc., sonicator model 150VT) for 15 min with a short burst of 20 s at 30 s intervals, keeping the sample on ice. The extraction process was repeated 3 times. The organic extracts were pooled and syringe-filtered (0.45 μm PTFE), dried over anhydrous sodium sulfate before a final syringe filtration. The organic solvent was evaporated under a gentle stream of dry N_2 gas. The mixture of total lipids, thus obtained, was weighed and reconstituted in 250 μL of chloroform–methanol (1:1, *v*/*v*) for chromatographic analyses.

High-performance thin-layer chromatography (HPTLC) of lipids

HPTLC glass plates (10 cm × 10 cm, Camag, Switzerland) precoated with fluorescent silica gel 60-F254, were prewashed with chloroform–methanol (1:1, *v*/*v*) and air–dried and activated at 120 °C for 20 min before use. The reconstituted total lipid extract was spotted on a freshly activated HPTLC plate, 2 cm above a

corner of the plate, and developed in chloroform–methanol–water mixture (75:25:5, by volume) until the solvent front reached 1 cm from top of the plate. The chromatogram, after development, was air-dried at room temperature for 10 min, and the chromatogram was redeveloped in the second direction at a 90° angle (i.e., 2D) in a second solvent mixture chloroform–methanol–water–glacial acetic acid (80:9:2:12, by volume) until the solvent front reached 1 cm from top of the plate. The plate was air-dried and stained with molybdophosphoric acid ($H_3[P(Mo_3O_{10})_4]$) (Sigma-Aldrich, USA) solution, 10 g dissolved in 100 mL absolute ethanol, *m*/*v*), followed by heating the plate at 80 °C for 2 min. The plate was briefly heated at 80 °C for 2 min to visualize PLs on a yellowish background.

FA profile in response to abiotic stress

Geobacillus gargensis was cultivated using seed cultures (50 mL) in cotton-plugged 250 mL Erlenmeyer baffled flasks at 60 °C in nutrient broth and under optimum growth conditions in a shaking incubator (120 r/min). The culture was aseptically transferred to 500 mL of fresh nutrient broth media in 2 L baffled flasks containing 500 mL of the growth medium. Variable abiotic stress was imposed while maintaining the optimum growth conditions (at 60 °C growth temperature, 10 h incubation time, 86 mmol/L NaCl, added in the growth medium at pH 7). The individually induced variable stress parameters were temperature (45, 55, 60, 65, 70 °C), incubation time (4.5 h, 24 h, 48 h, 6 days, 10 days), pH (4.5, 5.2, 6.3, 7, 8.1), salinity (14, 86, 150, 210, 340, 510 mmol/L NaCl), and anoxic conditions maintained under nitrogen gas and also with an addition of SDS at a concentration of 2 or 5 mmol/L. After exposure to the individual stress, the cells were centrifuged (3000 r/min (1070*g*) for 20 min) and thoroughly washed under aseptic conditions, with 50 mmol/L phosphate buffer, pH 7. Bacterial pellets were frozen at –20 °C for 24 h and then freeze-dried (Freezemobile and Bench Top freeze dryer with sentry microprocessor control, JHE VirTis Company, Inc., Gardiner, New York, USA), and the cells were securely and aseptically stored in screw-cap vials at 4 °C.

Trans-esterification of lipids

Freeze-dried and desiccated bacterial cells (100 mg) were suspended in 0.5 mL of boron trifluoride (BF_3) methanolic solution (Sigma-Aldrich, St. Louis, Missouri, USA) in screw-cap vials and sonicated for 15 min followed by heating at 50 °C for 20 min. The vials were cooled in ice, and 4 mL of distilled, cold hexane and distilled water (1:1, *v*/*v*) were added. The reaction mixture was vortexed, extracted 3 times with distilled *n*-hexane, and the organic extracts (6 mL) were pooled. The pooled organic extract was washed with cold saturated solution of NaCl (610.8 mmol/L). The washed organic layer was dried over anhydrous sodium sulfate, syringe-filtered, and the organic solvent was evaporated under a gentle stream of dry N_2 gas. The FAME mixture, thus obtained, was reconstituted in 250 μL of distilled *n*-hexane and analyzed by GC–MS.

GC analyses and identification of FAMEs

GC–MS interfaced with National Institute of Standards and Technology's (NIST) library database was used to record mass spectral data. Helium, as a carrier gas (He, purity grade 5, Kuwait Oxygen and Acetylene Co., Gulf Cryo, Kuwait), was used at a constant flow rate of 1.0 mL/min with an average velocity of 37 cm/s; the inlet pressure was maintained at 8.81 psi (1 psi = 6.894 757 Pa). The FAME mixture was resolved on a Varian capillary column VF-5ms (30 m by 0.25 mm inside diameter, 0.25 μm film thickness; Middelburg, Netherlands). The GC temperature program used for the separation of the FAME mixture was as follows: inlet temperature, 250 °C; splitless injection volume, 0.2 μL (syringe size, 2 μL); GC oven temperature program, 70 °C, hold 1 min, ramp 10 °C/min to 160 °C, hold 2 min (run time, 12 min), ramp 5 °C/min to 210 °C, hold 5 min (run time, 27 min), ramp 3 °C/min to 250 °C, and hold 15 min (total run time, 55 min). Identification of the FAMEs was carried out by comparison of the retention times, retention indices (mass finder software was used), and their mass spectral fragmentation pattern compared with the standards run under identical conditions. The MS was tuned daily with standard FAMEs solution.

Statistical analysis

Samples were analyzed in triplicate and arithmetic mean was taken. Standard deviation was calculated from the square root of data set variance. Significance of the results was measure by one-way ANOVA and Kruskal–Wallis test using Prism-5 software.

Results

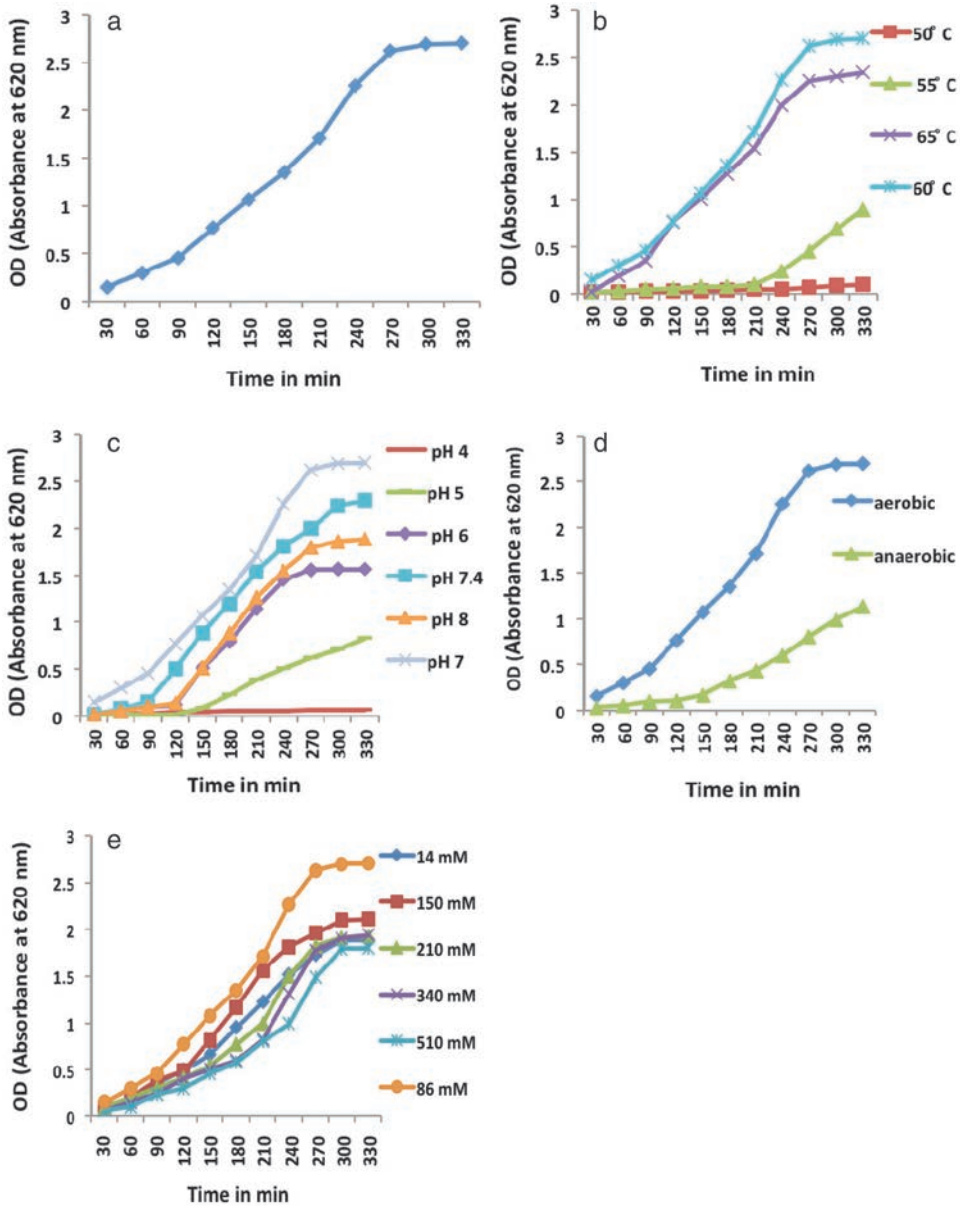
The growth curve for *G. gargensis*, cultured under standard conditions, is shown in Fig. 1*a*. The bacterium, under aerobic conditions, showed an exponential (log phase), fast and progressive growth at 60–65 °C, in 3.5–4.5 h incubation time, in the presence of 86 mmol/L NaCl at pH 7 (standard growth conditions). Temperature stress was imposed on the bacterium by its cultivation at 50, 55, 60, and 65 °C, and the growth curves are shown in Fig. 1*b*. As noted from the growth curve, 60 °C was the optimum growth temperature for the organism. The log phase of bacterial growth lasted for a period of 3.5–4.5 h. Since soil microbial communities are maintained at a certain soil pH, alkalinophilic and acidophilic stresses were imposed on *G. gargensis* by maintaining the bacterial growth at a 4.1–8.1 pH range. The growth curves at these pH values are shown in Fig. 1*c*. It was noted that *G. gargensis* had a good capacity to grow at a range of pH 5.2–8.1, but optimum growth was recorded at pH 7. The bacterial growth was studied under oxic and anoxic conditions, and the growth curves are shown in Fig. 1*d*. Since *G. gargensis* is an aerobe, it showed progressive growth under the oxic environment. The bacterial growth under anoxic conditions was poor with diminutive accumulation of biomass. Modifying salt concentration in the growth medium imposed an ionic and oxidative stress on the bacterial growth. Salt concentration in the growth medium was maintained at 14, 86, 150, 210, 340, and 510 mmol/L NaCl, and the growth curves are shown in Fig. 1*e*. The bacterium showed its best growth at a salt concentration of 86 mmol/L, indicating that the bacterium is a mild halophile.

SEM of *G. gargensis* cells under abiotic stress

The SEM results for *G. gargensis* cultured under normal and variable abiotic stresses are shown in Figs. 2*a*–2*l*. The incubation time had a profound effect on the formation of biofilm (EPS). At log phase (4.5 h) of growth, cells were healthy and dividing with minimum protruding EPS (Fig. 2*a*), whereas at death phase (after 10 days incubation) cells displayed an increased aggregation of EPS attachment with cell surface proteins, resulting in an unembellished biofilm formation (Fig. 2*b*).

In an alkaline growth medium, the bacterium displayed sluggish growth and a rough cell surface covered with EPS (Fig. 2*c*), whereas in the acidic environment (Fig. 2*d*), the EPS increased, covering most cells. High salt concentration (510 mmol/L) is a growth-limiting stress factor that resulted in the formation of additional EPS, making the bacterial surface rigid (Fig. 2*e*) compared with lower salt concentration (14 mmol/L), where cells appeared with less EPS and showed a relatively smooth surface (Fig. 2*f*). EPS, as a part of the biofilm formed at higher salt concentration, resembled a cocoon covering the cell surfaces. Bacterial cultures at low temperature (45 °C) showed very poor growth and a large amount of EPS formation (Fig. 2*g*). However, at higher temperature (70 °C), growth was normal and formation of EPS minimal (Fig. 2*h*). Bacterial growth was observed under both oxic and anoxic conditions, but under oxic conditions, the EPS formation was minimal cell number increased (Fig. 2*i*). Cell growth under anoxic conditions was poor and formation of EPS extensive (Fig. 2*j*). The addition of SDS at low concentration (2 mmol/L) showed reduced EPS formation and fewer cells (Fig. 2*k*), and

Fig. 1. Growth curves for *Geobacillus gargensis* under variable abiotic stresses. Effect of (*a*) growth under controlled conditions, (*b*) incubation temperature, (*c*) pH, (*d*) oxygen availability, and (*e*) culture salinity.



higher SDS concentration caused inhibition of bacterial growth and extended formation of EPS (Fig. 2*l*).

Effect of abiotic stress on total lipids

Total lipids were extracted from freeze-dried bacterial cells and their percentage yields were calculated. The temperature shock (45 °C) showed a drastic decrease by 46.1% in the accumulation of total lipids compared with a 6.8% yield under optimum bacterial growth temperature 60 °C (Fig. 3*a*). Incubation time also influenced the bacterial lipid accumulation. Thus, after 24 h of bacterial growth, the lipid accumulation was highest (7.6%), but it diminished by 1.71% at log phase of growth, declining to 4.57% in the cell lytic phase (Fig. 3*b*). Under gradient pH 4.1–8.1, formation of the total lipids amplified from 2.3% to 6.1% at pH 8.1, while the total lipid accumulation was 6.3% at neutral pH 7.0 (standard culture conditions, Fig. 3*c*). Salt stress was very critical for lipid accumulation, and it was salt concentration dependent. At lower salt stress (150 mmol/L), total lipids decreased by 18.8%, while at higher salt concentrations (510 mmol/L), the total lipids radically decreased by 13.1% (Fig. 3*d*), in comparison with bacterial culture under standard conditions (86 mmol/L salt). Lipid accumulation increased by 18.52% under oxic growth conditions compared with anoxic growth conditions. Addition of SDS at both concentrations reduced total lipid accumulation to 10.3%. Total lipid percentage extracted from *G. gargensis* under variable abiotic stress is shown in Figs. 3*a*–3*d*.

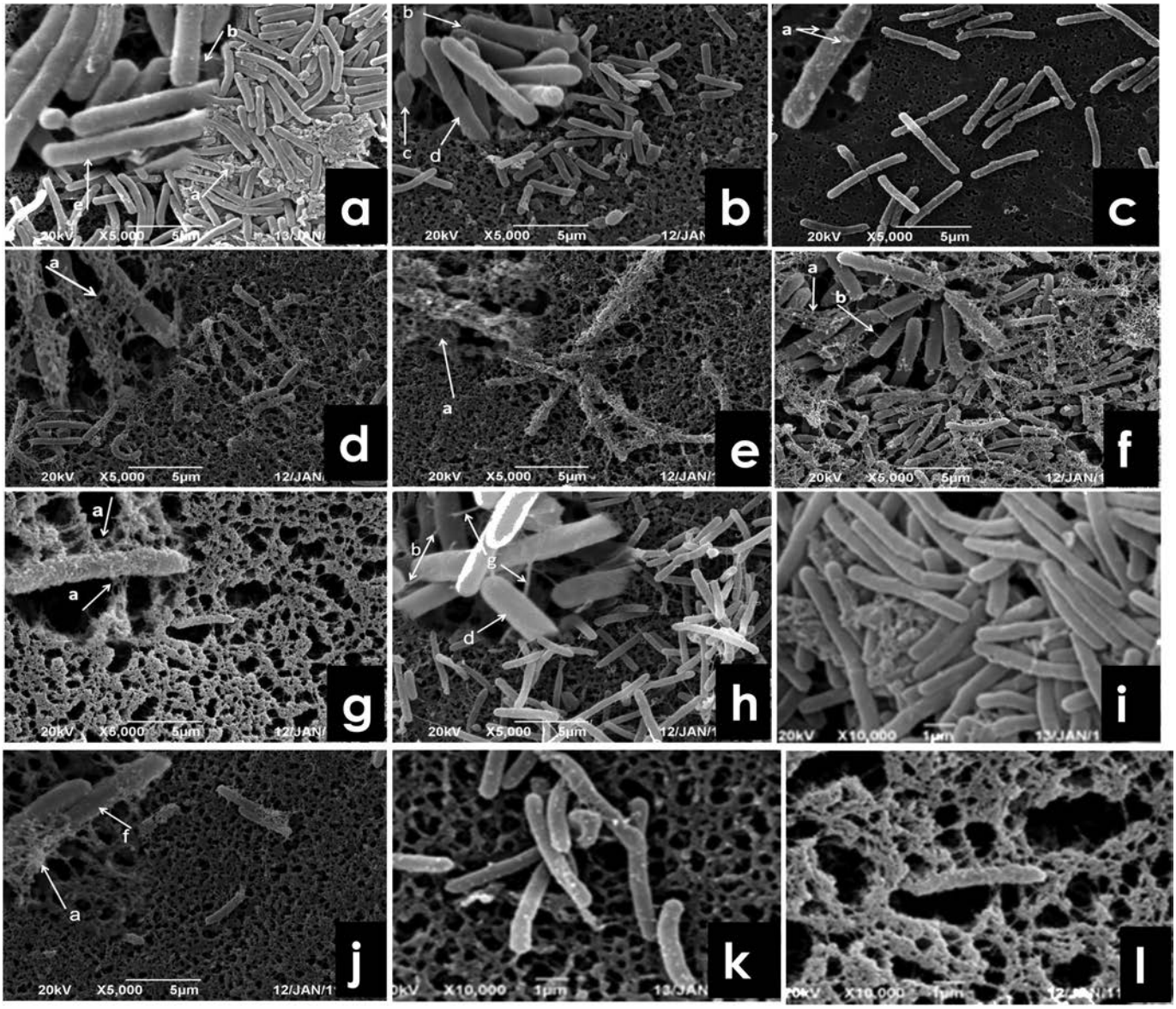
Geobacillus gargensis membrane PLs

Polar PLs were analyzed by 2-dimensional TLC using HPTLC plates. Five major PLs, namely phosphatidylcholine, phosphatidylethanolamine, cardiolipin, phosphatidic acid, and phosphatidylglycerol, were identified in bacterial cells cultured under normal growth conditions. The PL resolution on HPTLC plate is shown in Fig. 4. Scanning of the HPTLC plate revealed a progressive decrease in all PLs under all types of applied stresses.

Effect of abiotic stress on FAs profile

A mixture of standard bacterial FAMEs was resolved on a GC column resulting in 24 well-separated peaks (Fig. 5). The standard

Fig. 2. Scanning electron micrographs of bacterial cells. (*a*) Cells cultured under optimum growth conditions (4.5 h, 60 °C, pH 7.0, 86 mmol/L salt concentration, oxic conditions); (*b*) 10 days growth; (*c*) culture at alkaline pH 8.0; (*d*) culture at acidic pH 4.0; (*e*) culture in high salt concentration (510 mmol/L); (*f*) culture in low salt concentration (14 mmol/L); (*g*) culture at low temperature (45 °C); (*h*) culture at high temperature (70 °C); (*i*) culture under oxic conditions; (*j*) culture under anoxic conditions; (*k*) culture in 2 mmol/L SDS; (*l*) culture 5 mmol/L SDS. Arrows with letters show cells covered with or without extracellular polymeric substances.



bacterial FA mixture contained 10 saturated straight-chain FAs, 4 hydroxyl FAs, 4 saturated branched-chain FAs, 3 monoenoic FAs, 1 dienoic FA, and 2 cyclopentane FAs. The MS fragmentation of these FAs is shown in Table 1, and a representative GC resolution of FAMEs from *G. gargensis*, cultured under normal conditions, is shown in Fig. 6. A total of 13 FAMEs were identified from *G. gargensis*, cultured under standard culture conditions. *cis*- and *trans*-octadecenoate FAs were the only unsaturated FAs in the organism. These FAs were identified from their retention time (RT), retention indices, and MS fragmentation pattern (Table 2). In *G. gargensis* cultured under standard conditions, the ratio of the iso-FAs/anteiso-FAs was 4.08, the ratio of branched-chain FAs/straight-chain FAs (BCFAs/SCFAs) was 12.95, and the ratio of straight-chain saturated FAs/straight-chain unsaturated FAs (SCSFAs/SCUFAs) was 8.52.

A temperature shock to bacterial growth had a significant impact on the composition of FAs. Thus, the percentage yield of total BCFAs increased by 31.67% at an incubation temperature of 65 °C but drastically declined by 6.4% at lower temperature (45 °C). The lower incubation temperature of 45 °C resulted in a significant increase by 18.9% of iso-C16:0 FA, but its yield dropped by 25.1% with an increase in culture incubation temperature (65 °C). However, at 45 °C, the other iso-FAs, such as C15:0 and C17:0, ebbed by 27.5% and 23.5%, respectively. The yield of iso-C17:0 was amplified by 36.36%) with an increase in the incubation temperature. The total amount (% yield) of SCSFAs+SCUFAs showed a highly significant increase by 54.9% ($p < 0.01$) in response to low incubation temperature (45 °C), compared with the increase by 50.76% that was observed at higher incubation temperature (70 °C) (Fig. 7*a*). The ratio of SCSFA/SCUFA significantly ($p < 0.01$) decreased by 63.78% and 26.4% at incubation temperatures 45 and 70 °C, respectively, compared with the growth at 55, 60, or 65 °C (Fig. 7*a*). The ratio of iso-/anteiso-FAs remained almost constant at all growth temperatures (Fig. 7*a*). Surprisingly, the ratios of BCFAs/SCSFAs

Fig. 3. Accumulation of lipids in *Geobacillus gargensis* under variable abiotic stresses. Effect of (*a*) incubation temperature, (*b*) incubation time, (*c*) culture pH, and (*d*) culture salinity.

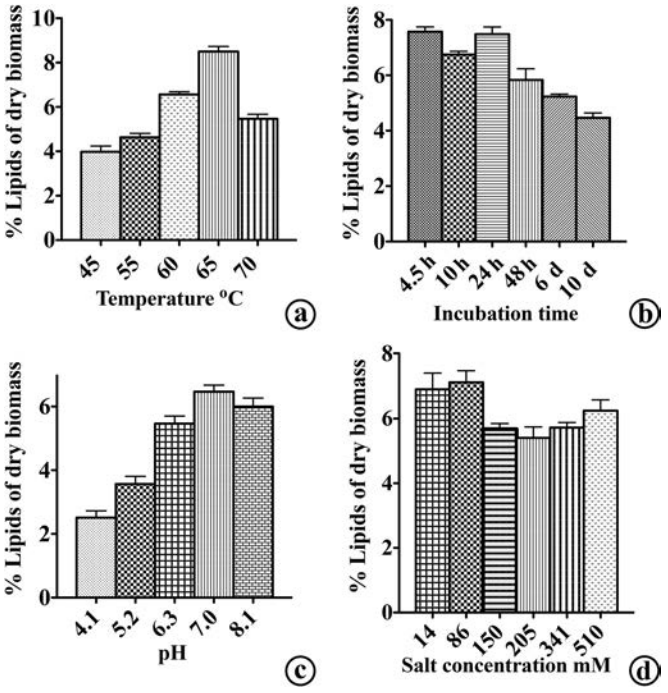
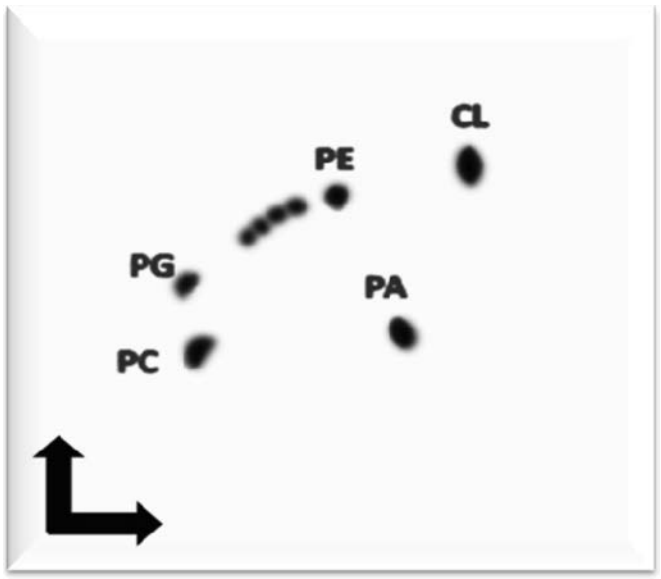


Fig. 4. Separation and identification of phospholipids by thin-layer chromatography. PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PA, phosphatidic acid; CL, cardiolipin.



and SCSFAs/SCUFAs declined significantly at 45 and 70 °C but remained almost constant at 55, 60, or 65 °C.

The influence of incubation period on *G. gargensis* constituent FAs was time-dependent. Thus, at an early phase of growth, an increase (6%) in the total BCFAs, a decrease (6.1%) in individual iso-C16:0, and an increase (~20.96%) in iso-C17:0 was observed. At 4.5 h incubation time, a significant increase of 29.23% ($p < 0.05$) in the SCFAs/SCUFAs ratio was observed, compared with the ratio obtained under standard culture incubation conditions. The ratio decreased at other incubation periods (Fig. 7*b*). This may be due to an increased synthesis of saturated FAs at early stages of bacterial growth that may be desaturated during increased growth periods. This fact may be supported by an increase in the ratio of BCFAs/SCSFAs (Fig. 7*b*).

The BCFAs/SCSFAs ratio significantly increased by 9.98% ($p < 0.05$) after 10 h of growth and significantly declined by 20.33% ($p < 0.03$) after 6 and 10 days of growth, compared with 4.5 h of growth (Fig. 7*b*). The ratio of total iso-FA/anteiso-FA displayed an increase of 7.2% in the early phases of bacterial growth (at 24 h incubation) compared with 4.5 h of incubation. This ratio non-significantly ($p \geq 0.05$) declined at all other incubation periods (Fig. 7*b*). The ratio of SCSFAs/SCUFAs also altered with time, with a significant increase ($p < 0.03$) of 29.85%, 27.55%, and 27.33%, respectively, at 4.5, 10, 24 h of bacterial growth. However, with increased incubation time (48 h, 6 days, 10 days), this ratio significantly ($p < 0.01$) decreased by 59.78% (Fig. 7*b*).

Variation in pH (Fig. 7*c*) had a pronounced damaging effect on the bacterial lipid FAs. In the present study, the highest accumulation of total lipids (6.8% ± 0.12%) was observed for growth media at neutral pH 7.0 (Fig. 3*c*) but declined at alkaline pH 8.1 (6.1% ± 0.19%). Thus, our results showed a decrease in iso-FA/anteiso-FA at pH 8.1 (Fig. 7*c*). Acidophiles are capable of growth at a pH range of 3.0–7.5 (optimum pH 4.5–5.0) but are sensitive to salt stress at 0.1% NaCl. *Geobacillus gargensis*, when cultured in growth medium with an acidic pH (pH 4.1, 5.2), showed a highly significant ($p < 0.01$) increase of 69.54% in the total SCSFAs and SCUFAs; however, the ratio SCSFAs/SCUFAs decreased along with a decrease in the BCFAs/SCSFAs ratio and a parallel increase in the iso-FA/anteiso-FA ratio (Fig. 7*c*). When cultured at an acidic pH of the culture, the percentage of total anteiso-C(17:0) and anteiso-C(15:0) highly significantly ($p < 0.01$) decreased by 47.86% and 59.18%, respectively, with a parallel increase in the total octadecenoic (C18:1; *cis* and *trans*) and total saturated FAs C(18:0) and C(16:0).

The appearance of C18:2 (*cis*-9,12) and the oxo-FA, methyl-12-hydroxy-octadec-9-enoate was only noticed when *G. gargensis* was cultured in growth medium with an acidic pH as compared with a neutral or alkaline pH, indicating biohydrogenation of C18:2 at acidic pH. However, at an alkaline pH, the BCFAs were observed as the prevalent FAs, with a 30% increase in BCFAs (increase in the ratio of BCFAs/SCSFAs). Among the BCFAs, the iso-C(16:0) showed a significantly ($p < 0.03$) higher increase (36.32%) in bacteria grown in the alkaline culture medium but only a 15.8% increase under acidic culture conditions. In pH adaptation, the balance of iso- and anteiso-FAs is more important than the degree of unsaturation of SCFAs. The maximum iso-/anteiso-FAs ratio was observed for acidic pH 4.1 and 5.2 culture medium (Fig. 7*c*), indicating lesser availability of 2-methylbutyrate, a precursor for the synthesis of anteiso-FAs. High-melting-point iso-FAs (BCFAs) may be required to maintain the membrane fluidity at acidic pH [17]. This was supported by the fact that the ratios SCSFAs/SCUFAs and BCFAs/SCFAs were only 1.5%–3.5% for acidic pH (4.1, 5.2) growth medium.

Variable salinity had dire consequences on the FAs composition of the bacterium. The concentration of all iso-FAs, including iso-C(15:0), iso-C(16:0), and iso-C(17:0), were salt concentration dependent. However, concentration of the total anteiso-FAs was inversely related to salt concentration. Total SCSFAs and SCUFAs significantly ($p < 0.05$) increased by 16.3% at all salt concentrations but nonsignificantly decreased (<1%) at the highest salt concentration (510 mmol/L). The ratio of SCSFAs/SCUFAs was highest at low salt concentration, which declined with an increase in the salt concentration (Fig. 7*d*). The total amount of BCFAs decreased in the range of 0.7%–2.2% at all salt concentrations. However, the ratio of iso-FAs/anteiso-FAs was higher at higher salt concentration (Fig. 7*d*).

Culturing the bacterium under anoxic conditions had the most noticeable consequences on the FAs component of the bacterium. Thus, BCFAs/SCSFAs, SCSFAs/SCUFAs, iso-/anteiso-FAs ratios decreased under anoxic culture conditions (Fig. 7*e*). The addition of

Fig. 5. Standard mixture of fatty acid methyl ester (FAME) resolution by gas chromatography on a Varian F4 capillary column. The temperature program and FAMEs identification is given in Materials and methods. Time scale is measured in minutes.

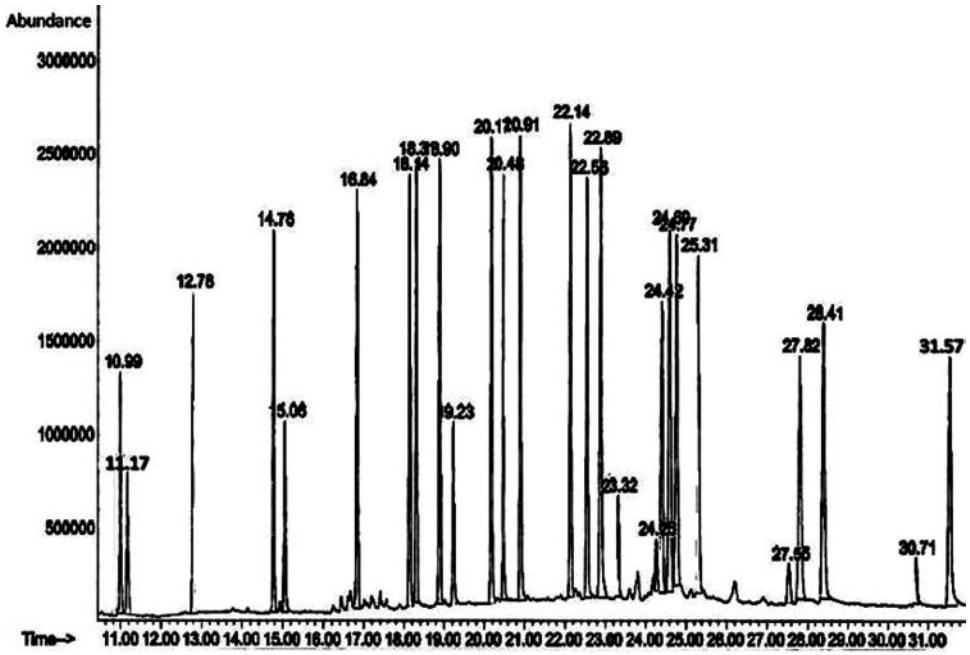
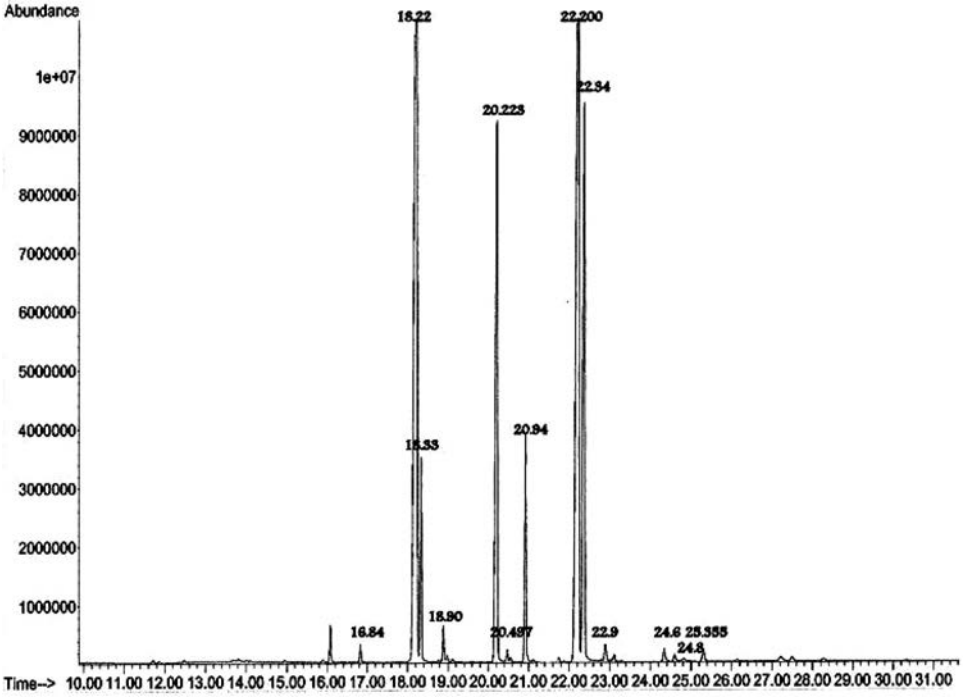


Fig. 6. Gas chromatography – mass spectrometry resolution of fatty acid methyl esters obtained from *Geobacillus gargensis* cultured under optimum conditions: 86 mmol/L NaCl for 10 h at 60 °C and pH 7.0. Time scale is measured in minutes.



SDS at both concentrations nonsignificantly affected FA composition of the organism and its addition showed no effect on the formation of EPS.

Discussion

Effect of abiotic stress on the formation of EPS

Geobacillus gargensis has a good capacity for adaptation to various stresses. However, extreme stresses cause formation of protective EPS that may contribute to its stress adaptation capacity. When making a network with the cell proteins through hydrogen bonds, EPS generates biofilm that plays an important role in the adaptation and (or) protection of the organism. The bacterial EPS is also a potential biosorbent for heavy metals from polluted water and environment. The entrapment is through the formation of complexes between EPS and heavy metals such as Cd(II); this leads to environmental bioremediation (Panwhichian et al. 2011).

Table 1. Mass spectral (MS) fragmentation of standard fatty acid methyl esters (FAMEs) resolved on a Varian F4 capillary column.

Peak No.	Retention time (min)	MS fragmentation (*m*/*z*)*	Molecular ion	FAME
1	10.99	M+. 200 (10), 169 (15), 157 (17), 87 (65), 74 (100; B), 55 (15)	200	C11:0 Undecanoate
2	11.17	M+. 202 (2), 143 (80), 90 (20), 83 (67), 69 (100; B), 55 (40)	202	2-OH-C10:0 2-Hydroxydecanoate
3	12.75	M+. 214 (10), 171 (20), 143 (25), 87 (70), 74 (100; B), 55 (18)	214	C12:0 Dodecanoate
4	14.84	M+. 228 (12), 185 (20), 143 (25), 87 (71), 74 (100; B), 55 (17)	228	C13:0 Tridecanoate
5	15.06	M+. 230 (7), 171 (98), 111 (30), 97 (100; B), 55 (59)	230	2-OH-C12:0 2-Hydroxydodecanoate
6	16.84	M+. 242 (18), 199 (31), 143 (33), 87 (72), 74 (100; B), 55 (20)	242	C14:0 Tetradecanoate
7	18.22	M+. 256 (15), 213 (21), 143 (25), 87 (79), 74 (100; B), 55 (19)	256	iso-C15:0 13-Methyltetradecanoate
8	18.33	M+. 256 (10), 199 (40), 87 (75), 74 (100; B), 69 (19), 55 (25)	256	anteiso-C15:0 12-Methyltetradecanoate
9	18.90	M+. 256 (17), 213 (20), 87 (70), 74 (100; B), 55 (16)	256	C15:0 Pentadecanoate
10	19.23	M+. 258 (7), 199 (90), 111 (49), 97 (73), 83 (70), 69 (100; B), 55 (53)	258	2-OH-C14:0 2-Hydroxytetradecanoate
11	20.22	M+. 270 (25), 227 (30), 143 (29), 129 (10), 87 (75), 74 (100; B)	270	iso-C16:0 14-Methylpentadecanoate
12	20.48	M+. 268 (14), 236 (50), 194 (41), 152 (32), 74 (37), 55 (100; B)	268	C16:1 (*cis*-9) *cis*-9-Hexadecenoate
13	20.94	M+. 270 (20), 227 (18), 143 (21), 87 (70), 74 (100; B), 55 (18)	270	C16:0 Hexadecanoate
14	22.14	M+. 284 (25), 241 (31), 199 (11), 143 (25), 87 (77), 74 (100; B), 57 (15)	284	iso-C17:0 15-Methylhexadecanoate
15	22.56	M+. 282 (3), 250 (49), 208 (24), 96 (60), 83 (62), 69 (81), 55 (100; B)	282	C17:0$^{\Delta}$ (all *cis*-9,10) *cis*-9,10-Methylenehexadecanoate
16	22.89	M+. 284 (35), 241 (27), 185 (11), 143 (30), 87 (79), 74 (100; B), 57 (13)	284	C17:0 Heptadecanoate
17	23.32	M+. 286 (17), 227 (35), 111 (33), 90 (50), 83 (79), 69 (82), 55 (100; B)	286	2-OH-C16:0 2-Hydroxyhexadecanoate
18	24.42	M+. 294 (35), 263 (25), 109 (40), 81 (98), 67 (100; B), 55 (60)	294	C18:2 (all *cis*-9,12) *cis*-9,12-Octadecadienoate
19	24.60	M+. 296 (11), 264 (80), 222 (48), 180 (30), 111 (35), 55 (100; B)	296	C18:1 (*cis*-9) *cis*-9-Octadecenoate
20	24.77	M+. 296 (14), 264 (76), 222 (50), 180 (30), 111 (41), 97 (75), 55 (100; B)	296	C18:1 (*trans*-9) *trans*-9-Octadecenoate
21	25.31	M+. 298 (49), 255 (28), 199 (12), 143 (30), 87 (75), 74 (100; B), 55 (20)	298	C18:0 Octadecanoate
22	27.82	M+. 310 (5), 278 (70), 236 (28), 111 (38), 97 (73), 83 (69), 55 (100; B)	310	C19:0$^{\Delta}$ (all *cis*-9,10) *cis*-9,10-Methyleneoctadecanoate
23	28.41	M+. 312 (51), 269 (30), 143 (27), 87 (78), 74 (100; B), 69 (13), 55 (20)	312	C19:0 Nonadecanoate
24	31.57	M+. 326 (54), 143 (32), 87 (80), 74 (100; B), 69 (14), 55 (22)	326	C20:0 Eicosanoate

*Relative abundance in MS is shown in parentheses. "B" indicates base peak. *m*/*z*, mass-to-charge ratio.

Temperature, salinity, pH, and availability of oxygen are also important factors that facilitate the formation of EPS. Thus, *Pseudoalteromonas* sp. strain SCSE709-6 isolated from deep-sea sediments is known to increase EPS with increased temperature and salinity (Zhou et al. 2013). The EPS from *Vibrio parahaemolyticus* with its emulsifying features is reported to be stable at temperatures as high as 250 °C (Kavita et al. 2011). In the present study, we noted that EPS formation in *G. gargensis* was temperature-dependent, and its network was more discernable at high-end temperatures of 65–70 °C, suitable for a thermophilic growth. The production of EPS may be responsible for the heat and mass transfer within the bioaggregate matrix that obstructs the bacterial cell wall pores, compromising cell plasticity and nutrient transport, facilitating cell adhesion, and profoundly inhibiting its biochemical metabolic pathways.

Effect of abiotic stress on lipid accumulation

Increasing the incubation period drastically reduced lipid accumulation in *G. gargensis* (Fig. 3*a*). Nutrient-poor medium is known to reduce the bacterial cell size and numbers, impacting the biomass, lipid accumulation, and its constituent FA (Lagha et al. 2012; Yao et al. 2012). Nutrient deficiency also results in an accumulation of other carbon or energy storage materials, leading to diminished lipid accumulation in bacterial membranes (de Eugenio et al. 2010). The EPS also play an important role by increasing production as lipopolysaccharides, augmenting cell adhesion with a diminution in lipid accumulation (Lerner et al. 2009).

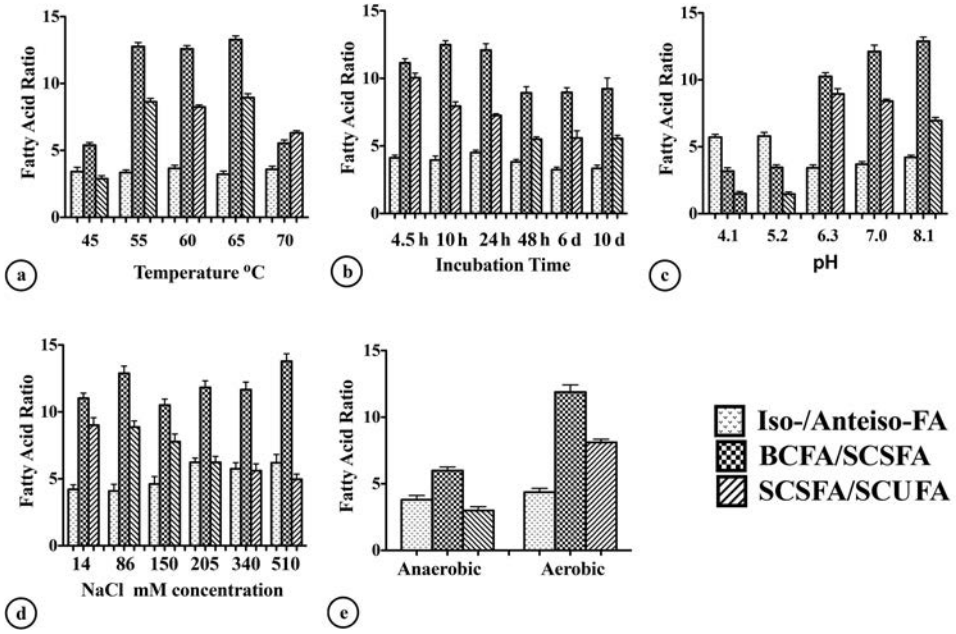
Increased incubation time of *G. gargensis* induced alterations in lipid components and their constituent FAs. This may have been due to an amplified formation of EPS that is known to be time- and pH-dependent (He et al. 2010; Patil et al. 2010; Feng et al. 2012). Our

Table 2. Bacterium cultured under standard conditions and their fatty acid with mass spectral (MS) fragmentation.

Peak No.	Retention time (min)	MS fragmentation (*m*/*z*)*	Molecular ion	FAME	% Peak area
1	16.84	M+. 242 (18), 211 (10), 199 (31), 143 (35), 87 (71), 74 (100; B), 55 (20)	242	C14:0 Tetradecanoate	0.40
2	18.22	M+. 256 (19), 213 (30), 143 (25), 87 (75), 74 (100; B), 55 (17)	256	iso-C15:0 13-Methyltetradecanoate	30.34
3	18.33	M+. 256 (10), 213 (20), 199 (35), 143 (25), 87 (75), 74 (100; B), 69 (19), 55 (25)	256	anteiso-C15:0 12-Methyltetradecanoate	3.43
4	18.90	M+. 256 (10), 225(8), 213 (15), 143 (19), 87 (70), 74 (100; B), 55 (16)	256	C15:0 Pentadecanoate	0.82
5	20.22	M+. 270 (30), 227 (35), 199 (10), 185 (11), 143 (32), 129 (10), 87 (75), 74 (100; B)	270	iso-C16:0 14-Methylpentadecanoate	12.80
6	20.48	M+. 268 (10), 236 (50), 194 (40), 152 (31), 74 (80), 96 (60), 83 (63), 96 (82), 55 (100; B)	268	C16:1 (*cis*-9) Hexadecanoate	0.40
7	20.94	M+. 270 (25), 239 (10), 227 (23), 199 (5), 185 (6),143 (25), 87 (75), 74 (100; B), 55 (19)	270	C16:0 Hexadecanoate	4.61
8	22.20	M+. 284 (35), 241 (40), 199 (15), 185 (14), 143 (32), 87 (80), 74 (100; B), 57 (14), 55 (20)	284	iso-C17:0 15-Methylhexadecanoate	28.00
9	22.34	M+. 284 (30), 255 (10), 241 (27), 277 (5), 199 (15), 185 (15), 143 (30), 129 (10), 97 (13), 87 (75), 74 (100; B), 75 (25), 55 (23)	284	anteiso-C17:0 14-Methylhexadecanoate	14.00
10	22.90	M+. 284 (25), 241 (20), 199 (10), 185 (10), 143 (27), 87 (75), 74 (10; B), 57 (18), 55 (18)	284	C17:0 Heptadecanoate	0.63
11	24.60	M+. 296 (10), 264 (60), 222 (45), 180 (25), 111 (32), 97 (60), 83 (69), 69 (80), 55 (100; B)	296	C18:1 (*cis*-9) *cis*-9-Octadecenoate	0.203
12	24.80	M+. 296 (10), 264 (52), 222 (30), 180 (28), 111 (33), 97 (64), 74 (69), 69 (77), 55 (100; B)	296	C18:1 (*trans*-9) Octadecanoate	0.20
13	25.30	M+. 298 (30), 255 (25), 199 (10), 143 (25), 87 (75), 74 (100; B), 55 (17)	298	C18:0 Octadecanoate	0.20

*Relative abundance in MS is shown in parentheses. "B" indicates base peak. *m*/*z*, mass-to-charge ratio.

Fig. 7. Effect of variable abiotic stresses on ratios of fatty acids. Effect of (*a*) incubation temperature, (*b*) incubation time, (*c*) incubation pH, (*d*) culture salinity, and (*e*) oxygen availability.



results show an amplification of EPS with both increased incubation period as well as variable pH (Figs. 2*c*–2*e*). Increased incubation periods result in nutrient removal, creating cell starvation, oxidative stress, consequently resulting in increased formation of EPS (Choi et al. 2001). The influence of temperature, time, and salinity on the formation of biofilm with the modulation of physiological parameters has been reported for the fish pathogen *Flavobacterium columnare* (Cai et al. 2013). In other cases, salinity shock on EPS formation has been documented (Ma et al. 2012; Qurashi and Sabri 2012).

Effect of temperature on FA ratio

Bacterial growth is influenced by alterations in its growth parameters. For example, bacterial growth is subject to abiotic stresses, such as hydrostatic pressure that changes the storage lipid composition (Grossi et al. 2010). Temperature-related

changes in membrane PLs have been reported (Bakholdina et al. 2007). Bacterial growth temperature has a direct influence on membrane protein expression, and membrane proteins can be upregulated or downregulated with temperature, which in turn, affects lipid metabolism and the constituent FAs. Thus, an increase in the bacterial growth temperature has been reported to result in an increase in the degree of saturation in FAs, leading to a decrease in unsaturated and cyclic FAs (Balamurugan and Dugan 2010). *Geobacillus gargensis* showed an increased synthesis of BCFAs and SCSFAs at 60–65 °C, indicating an activation of branched-chain alpha–keto acid reductase (BCKR). At lower culture temperatures, desaturases are upregulated with an increase in unsaturated FAs (Amiri et al. 2010). These changes reflect on the dynamic and physiological responses of *G. gargensis* subjected to variable temperature regimes. In thermophiles, at lower temperature (45 °C), it is known that BCKR, responsible for the synthesis of BCFA, activity is impaired, resulting in their diminished synthesis (Singh et al. 2008). A variation in cell adhesion due to EPS formation also affects the formation of FAs. Thus odd-carbon anteiso-C(15:0) and anteiso-C(17:0) are the principal FAs in floating cells, whereas in adhered cells, even-carbon SCSFAs such as 16:0 and 18:0 are produced (Singh et al. 2008). The ratio of BCFAs/SCSFAs and SCSFAs/SCUFAs was maximum at a temperature range of 55–65 °C, which is the optimum growth temperature of the organism (Fig. 7*a*). However, the ratio decreased at 70 °C, and the ratio of iso-/antiso-FAs remained almost unchanged at all tested temperatures.

Effect of incubation time on FAs ratio

A shorter incubation period (4.5 and 10 h) displayed a decline in BCFAs (iso-C(15:0)) at early phases of the bacterial growth. This may be due to a time-dependent and parallel increase in iso-C(17:0) observed in our studies. The decline in SCSFAs/SCUFAs ratio with increased incubation time (Fig. 7*b*) suggests that these FAs are used as energy reserves under nutrient starvation of bacteria, and after 10 days of incubation, a nonsignificant increase in the BCFAs/SCSFAs ratio may be due to a limited oxidation of the BCFAs for energy metabolism. Under nutritionally adverse conditions, the iso-/anteiso-FAs ratio remained almost constant, signifying impairment of the CYP4F gene for the metabolism of BCFAs and the metabolism of branched-chain amino acids under starvation conditions (Harwood and Canale-Parola 1981; Hardwick 2008).

Effect of pH on the FA ratio

In the formation of total lipids, culture pH variations also play an important role by modifying the cellular metabolic pathways leading to lipid metabolism. Acid stress is known to induce cell adhesion with increased EPS formation, controlling lipid synthesis and consequently SCSFAs accumulation (Gianotti et al. 2008). Supporting this, in the present study, the highest accumulation of lipids was observed in bacteria grown in neutral and alkaline pH media (Fig. 3*b*). This may be because growth media at these pH values offer a better environment for a decline in EPS formation and consequently cell adhesion resulting in enhanced anteiso-BCFAs synthesis (Singh et al. 2008). The ratio of BCFAs/SCSFAs was highest at pH 8.1, whereas the SCSFAs/SCUFAs ratio decreased, compared with pH 7.0; the iso-/anteiso FAs ratio remained unchanged (Fig. 7*c*).

Acidophiles are capable of growth at a pH range of 3.0–6.5 (optimum 4.5–5.0) but are very sensitive to salt stress, even at 0.1% NaCl. The main FAs produced by acidophiles are iso-C(15:0) and iso-C(17:0) (Pankratov et al. 2012). *Geobacillus gargensis*, when cultured at an acidic pH, predominantly synthesized SCSFAs and SCUFAs compared with BCFA. The formation of C(18:2), (*cis*-9,12), and the oxo-FA methyl-12-hydroxy-octadec-9-enoate was only noticed at acidic pH, indicating biohydrogenation of C(18:2) at an acidic pH (Alves et al. 2013). At an alkaline pH, the BCFAs were observed as the prevalent FAs and this was consistent with the reported literature (Giotis et al. 2007). In pH adaptation, the balance of iso- and anteiso-FAs is more important than the degree of saturation in SCSFAs. The iso-/anteiso-FAs ratio was observed highest at pH 4.1 and 5.2 (Fig. 7*c*), suggesting reduced availability of 2-methylbutyrate, a precursor for the synthesis of anteiso-FAs due to an inactivation of BCKR under acidic conditions (Kaneda 1988). High-melting-point iso-FAs may be required to maintain the membrane fluidity at acidic pH (Al-Khalaf et al. 2013). This was supported by the fact that the SCSFAs/SCUFAs and BCFAs/SCSFAs ratios were lowest at acidic pH but were maximum at alkaline pH (Fig. 7*c*).

Effect of salt concentration on the FAs ratios

Our results imply that *G. gargensis* has an ability to grow under a wide range of moderate salt concentrations, making the organism a mild halophile. Therefore, low salt concentrations are critical for growth of the organism. A salt-concentration-dependent decrease in the total lipid accumulation may reflect on the bacterial cells ion imbalance that may lead to desiccation, osmotic shock, and oxidative stress, resulting in growth inhibition. Peroxisomal membrane proteins, damaged in salt-induced oxidative stress, are known to affect the transport of long-chain FAs (Gibson et al. 2006). Results from our laboratory have previously indicated that salt stress adversely impacts total lipids in *G. kaustophilus* (Al-Khalaf et al. 2013). Salt-induced oxidative stress is known to downregulate BCKR, responsible for the synthesis of anteiso-BCFAs, thus lowering the synthesis of anteiso-BCFAs, which can be resumed by an addition of 2-methylbutyrate, a precursor for the synthesis of anteiso-BCFAs (Singh et al. 2008). Our results show that the even-carbon iso-C(16:0) FAs increased in concentration at both high and low salt stress. However, the odd-carbon iso-C15:0 and iso-C17:0 concentrations were salt-concentration-dependent. This may be due to an existence of 2 different metabolic pathways for even- and odd-carbon FAs producing acetyl-CoA and propionyl-CoA as end-products. Regulatory genes for both of these metabolic pathways are known to be diverse (Masiewicz et al. 2012). A reduction in the total amount of low-melting-point anteiso-FAs at various salt stresses accompanied by an increase in the high-melting-point iso-FAs and also SCUFAs is probably required to maintain cell membrane plasticity to combat ionic flux. The iso-/anteiso-FAs ratio was consistently elevated with an increase in salt concentration, which shows that decreasing, low-melting-point anteiso-FAs are required to raise cell rigidity against ionic salt strength. Whereas an increase in the BCFAs/SCSFAs ratio presumably indicated the sluggish metabolism of the organism, favoring synthesis of branched-chain FAs at increasing salt stress. The decreasing SCSFAs/SCUFAs ratio (Fig. 7*d*) with increasing salt concentration was indicative of activation of desaturases at higher salt concentration to produce additional SCUFAs. SCUFAs are known to be essential for suitable function of the cell membranes to contend with salt stress and for cell signaling (Zhang et al. 2012).

Anoxic conditions proved to be harmful for the organism, and accumulation of lipids was favorable under oxic growth conditions (Fig. 7*e*). The synthesis of long-chain FAs C(16:0) increased with a parallel decrease in BCFAs under anoxic growth conditions. Under these conditions the percentage of straight-chain saturated and unsaturated FAs increased more than 50%, indicating inactivation of BCKR under anoxic conditions. Our results show *G. gargensis* has the ability to grow under a variety of abiotic stress conditions with regulation in its membrane lipid synthesis. The ratio of iso-/anteiso-FAs in membrane lipids plays a crucial role in *G. gargensis*' adaptation to abiotic stress. The formation of EPS under different stresses also helps in adaptation of the organism.

The bacterial culture under oxic conditions imposed oxidative stress on the organism that again showed amplified formation of EPS. However, degradation of biofilms under both oxic and anoxic conditions has also been reported (McKew et al. 2013). Our results provide ample evidence to demonstrate that under all abiotic stress conditions, EPS was formed and its level was dependent on

the type and duration of the stress. The formation of EPS was studied in the presence of 2 concentrations of the surfactant SDS, and it was concluded that SDS shock presumably inhibited the formation of EPS as well as total lipids and their constituent FAs, at both concentrations of SDS (Adamowicz et al. 1991; Simoes et al. 2005). This study indicates both chemical and physical stress on *G. gargensis* result in its adaptation by alterations in membrane plasticity by adjusting membrane lipids and FA composition.

Conclusions

Membrane lipids and their FA composition are sensitive to pH, salt concentration, and temperature of the bacterial growth media. Formation of EPS may play an important role in bacterial adaptation under abiotic stress.

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