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African Journal of Microbiology Research

*Full Length Research Paper*

# Isolation and characterization of thermophilic bacteria from different habitats and their assessment for antagonism against soil-borne fungal plant pathogens

**Rajashree R. Pawar* and S. G. Borkar**

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**Three different biomaterials *viz.*, boiled cow milk, compost manure and tomato rhizospheric soil were found as habitats of the thermophilic antagonistic bacteria. The isolated bacteria were able to grow satisfactorily at thermophilic temperature range (>55°C). Based on morphological, biochemical and physiological characters, the bacterial isolates were identified as *Bacillus licheniformis* (boiled cow milk), and *Bacillus stearothermophilus* (compost manure and tomato rhizospheric soil). All the three thermophilic bacterial isolates exhibited strong antagonism against tested soil-borne fungal plant pathogens in order of *B. lechaniformis* (inhibition zone of 67.67 mm against *R. bataticola*) > *B. stearothermophilus* from compost manure (51.67 mm against *R. solani*) > *B. stearothermophilus* from tomato rhizospheric soil (38.33 mm against *P. aphanidermatum*). The ability to tolerate high temperature (>55□C), pH (6-8) and salt concentrations (up to 8%), and antibiotic resistance properties of the antagonistic thermophilic *Bacillus* isolates may hold them as potential biocontrol candidates, especially under stressed rhizosphere environments where other biocontrol agents fail. However, the results need further confirmation under field conditions where these bioagents will be applied in a formulated form.**

**Key words:** Antagonism, antibiotic sensitivity, biocontrol activity, soil borne pathogens, thermophilic bacteria.

## INTRODUCTION

Plant diseases caused by fungi, bacteria and viruses are the major factors limiting the agricultural productivity, reducing the crop yields to the tune of 16% globally (Oerke, 2006). The soil-borne disease complex consisting mainly of damping off, root rot, collar rot and wilts causes more than 50% losses in crop production (Biswas and Das, 1999). Globally, the chemical fungicides like metalaxyl, captan, benomyl, chlorothalonil, copper oxychloride and many more are being used desperately for the control of soil-borne crop diseases caused by pathogenic genera *viz.*, *Pythium*, *Fusarium, Phytophthora, Rhizoctonia* and *Sclerotium* (Rao et al., 2007; Wightwick et al., 2010). However, risk of ground water pollution affecting the quality of life, destruction of non-target beneficial soil microflora, deleterious effects on mycorrhizal associations, depletion of soil nutrient

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dynamics, especially of nitrogen are some of the deleterious effects associated with excess chemical use for the control of soil-borne plant pathogens (Georgieva et al., 2002; Van-Zwieten, 2004; Rao et al., 2007; Wightwick et al., 2010). Under such circumstances, biological control assumes critical importance in crop disease management, especially for soil-borne fungal diseases.

Antagonistic bacteria are the ideal candidates for biocontrol of plant diseases of economically important crops; for example *Bacillus subtilis* GB-03 against *F. oxysporum f. sp. vasinfectum* and *R. solani* in cotton (Brannen and Backman, 1994), *Bacillus cereus* against *Phytopthora sojae* in soybean (Osburn et al., 1995). However, failure of bacterial antagonists to survive and accord long term disease control under environmental stress conditions is a major hindrance to the adoption of biocontrol strategies for management of soil borne plant diseases (Hoitink and Boehm, 1999). The antagonistic bacteria that could tolerate extreme environmental conditions and offer efficient control of fungal plant pathogens are supposed to be good alternative for plant disease management. Being endospore formers, thermophilic bacteria can tolerate heat and resist themselves from desiccation under high temperature stress, a feature makes them an attractive candidate for biocontrol of plant diseases (Rao et al., 2007; Wightwick et al., 2010). They exhibit excellent seed and root colonising ability, thereby suppressing the growth of fungal pathogens effectively (Kim et al., 1997). Besides, they are abundantly present in rhizospheric soils as saprophytes which make them as ideal biological control agents for soil-borne plant diseases.

The occurrence of thermophilic bacteria has been reported from various biomaterials like milk and milk products (Ruckert et al., 2004; Ronimus et al., 2006), compost manure (Miyatake and Iwabuchi, 2005), and crop rhizospheric soils (Sao Paulo, 2007; Santana et al. 2013). However, their use as potential bioagents for the control of soil-borne fungal plant pathogens has been less explored. The basic aim of the present study was to isolate and characterize the thermophilic bacteria from three different biomaterials *viz*., boiled cow milk, compost manure and tomato rhizospheric soil and to evaluate their antagonistic potential against important soil borne fungal pathogens for possible introduction as biocontrol agents in plant disease management. Our study helped in identifying the promising candidate isolates of thermophiic bacteria which can further be up scaled for commercial bioformulation for control of destructive soil borne crop diseases.

## MATERIALS AND METHODS

### Sample collection

The samples of boiled cow milk and compost manure were obtained from the Cattle Improvement Scheme, Department of Animal Science and Dairy Science, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri. The rhizospheric soil of tomato crop was collected from experimental field of the Vegetable Improvement Scheme of Department of Horticulture, MPKV, Rahuri, Maharashtra, India. The temperatures of the sources at the time of the sampling were recorded as 81, 69 and 40°C for boiled cow milk, compost heap and tomato rhizospheric soil, respectively. The samples were collected in sterile polybags and immediately brought into the laboratory for isolation.

### Isolation of thermophilic bacteria and determination of thermal death points

The bacteria from collected samples were isolated on Nutrient Agar (NA) medium (Himedia, Mumbai, India) following enrichment culture technique (Allen, 1953). The 250 ml conical flasks containing 10 g of sample and 90 ml sterile nutrient broth were homogenised in orbital shaker at 150 rpm and were heat shocked by placing in water bath at 80°C for 10 min. The suspensions thus obtained were serially diluted. The sterilie NA plates were inoculated with 1ml of solution from serial dilution of $10^7$ using spread plate technique (), and were incubated at 55°C for 24 h. The distinct single colonies were subcultured onto freshly prepared manganese agar slants to facilitate further growth. The pure cultures of bacterial isolates were subjected to different temperatures ranging from 5 – 80□C for determination of thermal death points and survival at higher temperatures. For temperatures between 5 – 65°C, the bacterial isolates were tested using nutrient agar whereas for hyper thermophilic range (70 - 80°C) nutrient broth was used (Seeley and Vandemark, 1970). After incubation for 24 h, the NA plates were observed for growth pattern of bacterial isolates at respective temperatures while test tubes with NB were observed for turbidity.

### Identification of thermophilic bacterial isolates

Overnight grown cultures of the isolated microorganisms were examined under microscope for colony morphology, cell shape, size, arrangement and motility. The gram staining and endospore staining were performed as per the standard procedures mentioned in Bergey's manual of determinative bacteriology (Snaeth, 1986). The isolates were subjected to biochemical tests *viz*., catalase test, oxidase test, acid and gas production, casein hydrolysis, gelatin liquefaction, starch hydrolysis, citrate utilization, nitrate reduction, hydrogen sulfide production, indole production, urease activity and methyl Red-Voges Proskeur (MR-VP) tests by following the standard procedures given by Seeley and Vandemark (1970) and Cappuccino and Sherman (1987).

### Sensitivity to the concentrations of pH, NaCl and antibiotics in growth media

The effects of various concentrations of pH (2-10), NaCl (1-10%) and antibiotics on growth and sporulation of thermophilic bacterial isolates were determined by inoculating the NA slants with overnight cultures of thermophilic *Bacilli* and incubating at 50□C for 48 h (Gulati et al., 2007; Singh et al., 2010). Sensitivity to antibiotics *viz*., streptocyclin, streptomycin sulphate, bacterianashak and cyclohexamide with different concentrations (ppm) was evaluated (Imanaka et al., 1981). Growth pattern of bacterial isolates on different concentrations of pH, NaCl and antibiotics were characterised as no growth (-), slow growth (+), moderate growth (++), profused growth (+++) and very profused growth (++++).

### *In vitro* screening for antagonism against soil borne fungal pathogens

The thermophilic bacterial isolates were screened for antagonistic

**Table 1.** Screening of bacterial isolates for thermotolerance.

| Test temperature (°C) | *Growth pattern of bacterial isolates from | | |
|---|---|---|---|
| | Boiled cow milk | Compost manure | Tomato rhizospehric soil |
| | **Thermophilic range** | | |
| 80 | + | - | - |
| 75 | ++ | - | - |
| 70 | +++ | ++ | ++ |
| 65 | ++++ | ++ | ++ |
| 60 | ++++ | ++ | +++ |
| 55 | ++++ | +++ | ++++ |
| 50 | ++++ | ++++ | ++++ |
| | **Mesophilic range** | | |
| 45 | ++++ | ++++ | ++++ |
| 40 | ++++ | ++++ | ++++ |
| 35 | ++++ | +++ | +++ |
| 30 | ++++ | +++ | +++ |
| 25 | ++++ | ++ | +++ |
| | **Psychrophilic range** | | |
| 20 | ++ | + | + |
| 15 | + | + | + |
| 10 | - | - | - |
| 5 | - | - | - |

*Growth patterns: -, no; +, slow; ++, moderate; +++, profuse; ++++, very profuse.

activity against major soil borne fungal pathogens *viz.*, *Pythium aphanidermatum*, *Fusarium oxysporum* F. sp. *ciceri*, *Rhizoctonia bataticola, Sclerotium rolfsi, Fusarium oxysporum F. sp lycopersici* and *Rhizoctonia solani*. The test fungal plant pathogens used in present study were freshly isolated from the disease samples collected from experimental fields of Mahatma Phule Krishi Vidyapeeth, Rahuri. A dual culture technique (Morton and Stroube, 1955) was used for *P. aphanidermatum* and *S. rolfsi*, whereas seeding and disc assay method (Besson et al., 1978) was used of the rest of the pathogens. The thermophilic bacterial isolates exhibiting zone of inhibition against test pathogen were screened as potential antagonist. Morphological characters of approaching hyphae were observed every day under light microscope (400 IX70-S1F2, Olympus Optical Co. Ltd., Tokyo, Japan) and images were recorded with a digital camera (CAMEDIA C-3040 Zoom, Olympus Optical Co. Ltd.).

## RESULTS AND DISCUSSION

### Occurrence of thermophilic bacteria in various biomaterials

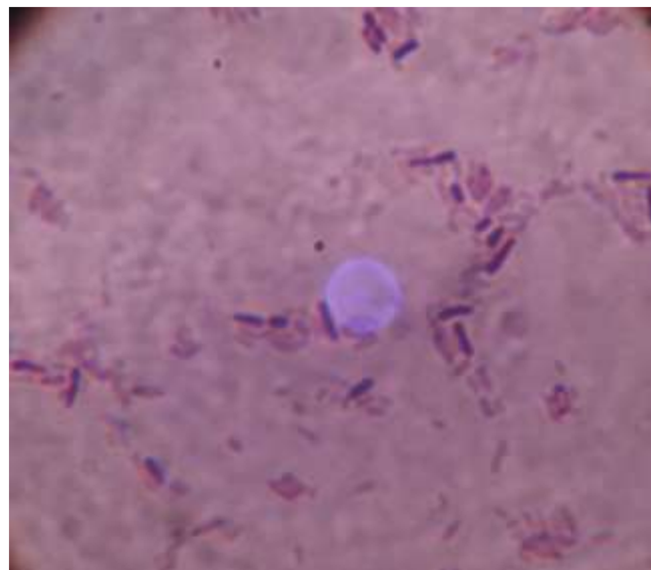
The profuse growth of bacterial colonies after incubation at 55°C for 48 h confirmed the occurrence of the thermophilic bacteria in all the three biomaterials that is boiled cow milk, compost manure and tomato rhizospheric soil. All the three isolates were able to sustain highly thermophilic temperature range (70°C) thereby indicating their thermophilic nature. The thermal minima and maxima for all three thermophilic bacterial isolates were 10 and 80°C, respectively. The highest thermal death point (TDP) was observed in bacterial isolate from boiled cow milk (80°C), whereas the TDPs for bacterial isolates from compost manure and tomto rhizospheric soil were both at 75°C. The bacterial isolates could grow moderately at temperatures between 15-20°C. The favourable temperature range for bacterial isolate from boiled cow milk was broad (25-65°C) compared to bacterial isolate from compost manure and tomato rhizosperic soil (40-55°C) (Table 1). Our results are in line with the work of earlier researchers who also reported boiled cow milk, compost manure and crop rhizospheric soils as habitats for the thermophilic bacteria. Nakanishi (1963) isolated *B. licheniformis* from milk samples heat treated at 120°C. Janstova and Lukasova (2001) isolated the *Bacillus* strains from raw milk and farm environment for thermo resistance which grew at the temperature range of 95-135°C. Fujio and Kume (1991) found that thermophilic strains of bacteria *B. stearothermophilus* and *Thermus* sp. isolated from compost were able to grow at optimum temperature range 60-65°C. Miyatake and Iwabuchi ((2005) investigated the enzymatic activity and species diversity of thermophilic bacteria in cattle manure compost at 54, 60, 63, 66 and 70°C, which were dependent on composting temperature. They observed the highest level of thermophilic bacterial activity at 54°C. Xiao et al. (2011) observed an increased biomass of thermophilic

bacteria due to continuous thermophilic composting (CTC) with incubation at 30, 40 and 50°C and concluded that CTC might have increased biomass of thermophilic bacteria, especially *Bacillus* spp. Nunes de Souza and Martin (2001) reported that the optimum growth temperature of a thermophilic bacterium isolated from a soil sample in Rio de Janeiro, Brazil was 55°C whereas upper temperature threshold for growth was around 70°C after which no growth was observed. Gulati et al. (2007) reported that thermophilic strain of *Bacillus laevolacticus* isolated from the rhizospheric soil of fenugreek plant (*Medicago falacata*) was optimally active at 70°C.

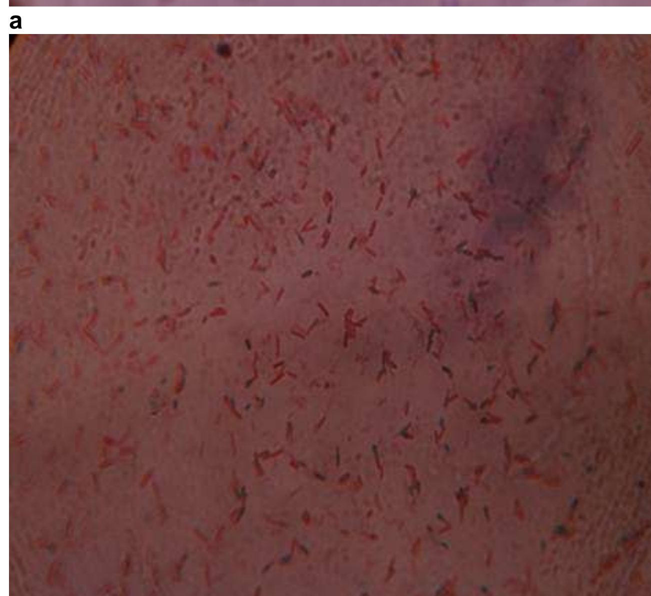
### Characterization and identification of thermophilic bacteria

The microscopic observations of thermophilic bacteria revealed that, all the three isolates were gram +ve, motile rods and capsule formers with endospores produced terminally (Figure 1). On nutrient agar (NA) medium, bacterial isolate from boiled cow milk produced creamy yellow, circular colonies with smooth glistening surface, raised elevation and smooth edges. Similarly, the colonies of bacterial isolates from compost manure and tomato rhizospheric soil were creamy white, flat with no elevation, rough and dry surface and with wavy edges. The characters observed were typical of the family *Bacillaceae* and were similar to those reported in literature (Nunes de Souza and Martin, 2001; Esikova et al., 2002; Pathak and Rekadwad, 2013).

The biochemical tests indicated that all the three thermophilic bacterial isolates were strict aerobes, catalase positive, oxidase positive, and were able to produce the acid from glucose, fructose, lactose and sucrose, except bacterial isolate from tomato rhizospheric soil which did not produce acid from lactose (Figure 2). None of the isolates could produce gas from glucose. The tests of casein hydrolysis and starch hydrolysis (Figure 3), and gelatin liquefaction were positive for all three isolates. The test of citrate utilization was positive only for the bacterial isolate from boiled cow milk (Figure 4). The other biochemical tests like $H_2S$ production, indol formation and urease activity were negative for all three thermophilic isolates. The test of methyl red was positive for all the three bacterial isolates whereas the Voges-Proskaer test was positive only when pH of the growth medium was less than 6.0. The biochemical characterization revealed that all the three thermophilic isolates are distinctly different from each other (Table 2). Our results are in larger agreement with literature reports. Nunes de Souza and Martin (2001) reported that thermophilic bacterium isolated from a soil sample in Rio de Janeiro, Brazil was strictly aerobic and catalase +ve. Fujio and Kume (1991), Akanbi et al. (2010) and Panda et al. (2013) also identified the thermophilic strains of bacteria as *Bacillus* sp. based on conventional gram straining technique, physiological and biochemical characterizations. Singh et al. (2010) reported that thermophilic *Bacillus cereus* SIU1 strain isolated from slightly alkaline soils of Uttar Pradesh was strict aerobe with positive catalase and oxidase activity and was able to hydrolyze casein and gelatin. All these reports are closely in agreement with present findings indicating that our thermophilic antagonistic bacterial isolates were of *Bacillus* sp.



a



b

**Figure 1.** Gram +ve rods of thermophilic bacteria isolated from tomato rhizospheric soil (a) and rods showing endospores terminally (b).

All the three thermophilic bacterial isolates preferred a pH range between 6.0-8.0, at which profuse growth was observed, even though they could grow at a wider pH range between 4-10. None of the isolates preferred highly acidic (2.0) and highly alkaline (12.0) pH range

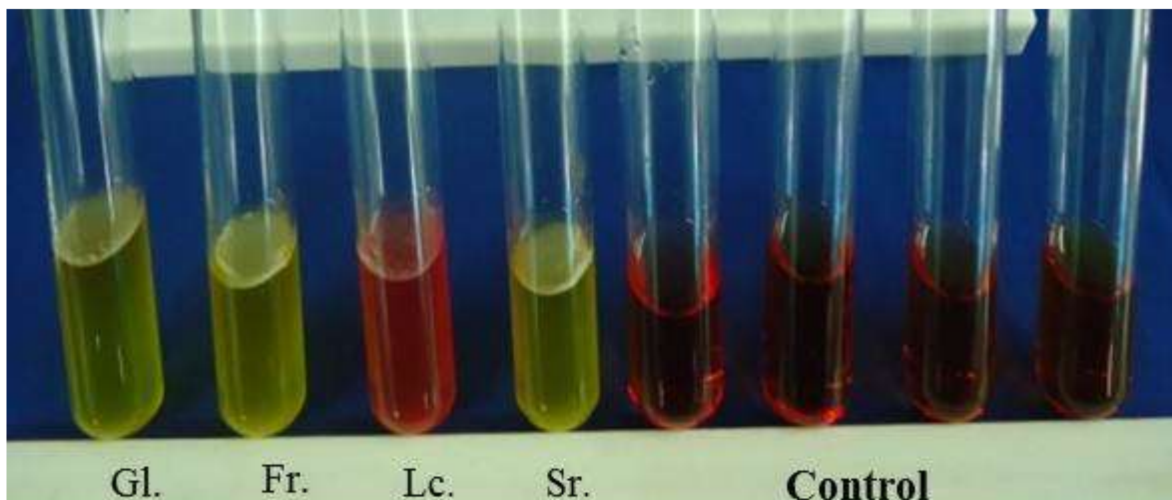



**Figure 2.** Acid production test in phenol red carbohydrate broth for thermophilic bacterial isolate from tomato rhizospheric soil. Gl = Glucose; Fr = Fructose; Lc = Lactose; Sr = Sucrose.

**Figure 3.** Starch hydrolysis test for isolate from compost manure. Formation of clear hollow zone around bacteria upon smearing with Gram's Iodine on starch agar plates indicates that bacteria are starch hydrolysis +ve.

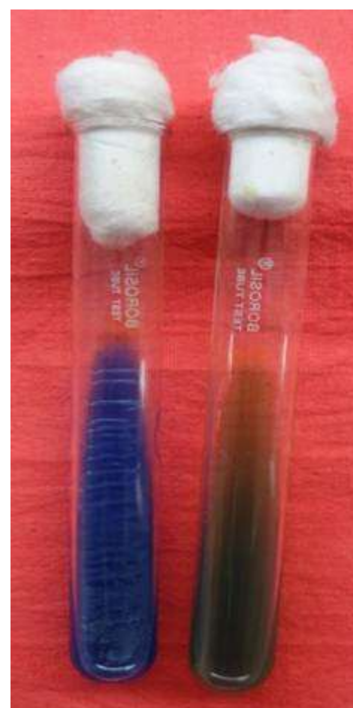


**Figure 4.** Citrate utilization test for thermophilic bacterial isolate from boiled cow milk. T = treatment; C = control. Change in colour from green to purple in Simmons's citrate slant indicates citrate +ve reaction.

(Table 3). Our results are closely in agreement with earlier reports on pH tolerance of thermophilic antagonistic bacteria. Nunes de Souza and Martin (2001) observed the optimum growth of a thermophilic bacterium from a soil sample in Rio de Janeiro, Brazil at pH 7.0. Gulati et al. (2007) reported that thermophilic strain of *Bacillus laevolacticus* isolated from the rhizospheric soil of fenugreek plant (*Medicago falacata*) was optimally active at pH between 7.0 - 8.0. Singh et al. (2010) reported that thermophilic *Bacillus cereus* SIU1 strain isolated from slightly alkaline soils of Uttar Pradesh grew over a wide range of pH between 5.0 -12.0.

All the three thermophilic bacterial isolates from

**Table 2.** Identification of antagonistic thermophilic bacterial isolates based on morphological and biochemical characterization.

| Bacterial isolates from | Morphological characters | | | Biochemical characters | | | | | | | | | | | | | | | | | | | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Carbohydrate fermentation | | | | | | Hydrolysis | | | | | | | | | Voges - Proskaer | | |
| | Rod shaped | Endospore produced | Stain Gram +ve in young cultures | Catalase | Oxidase | Glucose | Fructose | Lactose | Sucrose | Gas from glucose | Strict aerobes | Casein | Gelatin | Starch | Citrate utilization | Nitrate reduction | H2S production | Indol production | Urease activity | Methyl red | pH < 6.0 | pH > 7.0 | |
| Boiled cow milk | + | - | + | + | + | + | + | + | + | - | + | + | + | + | + | + | - | - | - | + | + | - | *Bacillus licheniformis* |
| Compost manure | + | + | + | + | + | + | + | + | + | - | + | + | + | + | - | + | - | - | - | + | + | - | *Bacillus stearothermophilus* |
| Tomato rhizospehric soil | + | + | + | + | + | + | + | - | + | - | + | + | + | + | - | + | - | - | - | + | + | - | *Bacillus stearothermophilus* |

**Table 3.** Effect of pH of nutrient medium on growth of thermophilic bacterial isolates.

| pH | *Growth pattern of bacterial isolates from | | |
|---|---|---|---|
| | Boiled cow milk | Compost manure | Tomato rhizospehric soil |
| 2 | - | - | - |
| 4 | +++ | +++ | +++ |
| 6 | ++++ | ++++ | ++++ |
| 8 | +++ | ++++ | ++++ |
| 10 | ++ | ++ | ++ |
| 12 | - | - | - |

*Growth patterns: -, No; +, slow; ++, moderate; +++, profuse; ++++, very profuse.

present study were able to tolerate salt concentration as high as 8.0%; however the sensitivity to NaCl concentrations differ significantly among the three isolates (Table 4). The bacterial isolate from boiled cow milk was comparatively more sensitive to NaCl concentration which grew profusely only up to 3.0 % salt concentration, whereas the other two isolates grew very profusely up to 5% salt concentration. Very slow growth was observed in the bacterial isolates from compost and tomato rhizospwhric soil at 9.0% NaCl concentration, whereas the isolate from boiled cow milk failed to grow at this concentration. None of the three thermophilic bacterial isolates were able

**Table 4.** Effect of different NaCl concentrations on growth of thermophilic bacterial isolates.

| NaCl concentration (%) | Growth pattern of bacterial isolates* | | |
|---|---|---|---|
| | Boiled cow milk | Compost manure | Tomato rhizospehric soil |
| 1 | ++++ | ++++ | ++++ |
| 2 | ++++ | ++++ | ++++ |
| 3 | ++++ | ++++ | ++++ |
| 4 | +++ | ++++ | ++++ |
| 5 | +++ | ++++ | ++++ |
| 6 | ++ | +++ | +++ |
| 7 | ++ | +++ | ++ |
| 8 | ++ | ++ | ++ |
| 9 | - | + | + |
| 10 | - | - | - |

*Growth patterns: -, No; +, slow; ++, moderate; +++, profuse; ++++, very profuse.

to grow at 10.0% NaCl concentration. Our results on high salt tolerance in antagonistic thermophiles suggest possible utilization of these bacteria as effective biocontrol agents for soil borne fungal pathogens, especially in saline and alkaline conditions. The literature reports exist on NaCl tolerance of thermophilic antagonistic bacteria. Elnasser et al. (2007) reported that thermophilic bacterial strain *Bacillus justea* I isolated from hot springs of Jordan grew optimally at NaCl concentration of 0.5%. The range of NaCl tolerance observed in our study is comparatively much higher than reported by Elnasser et al. (2007), which may be due to dissimilar strains. Singh et al. (2010) reported that thermophilic *B. cereus* SIU1 strain isolated from slightly alkaline soils of Uttar Pradesh was highly halotolerant, which was able to grow in the presence of 0.0-10% NaCl. These reports are in line with present findings with respect to salt tolerance of thermophilic *Bacillus* sp.

The antibiotic sensitivity of three thermophilic isolates varied for each of the antibiotic group tested. The isolate from boiled cow milk could tolerate concentrations of streptocyclin and streptomycin sulphate as high as 500 ppm, whereas the other two isolates could tolerate only up to 50 ppm. The isolate from tomato rhizospheric soil was more resistant to *Bacterianashak* (a bactericide) and could tolerate up to 1000 ppm, whereas the remaining two were able to tolerate only up to 500 and 250 ppm, respectively. The isolate from boiled cow milk (*B. licheniformis*) was less sensitive to cyclohexamide and could grow at concentration as high as 7000 ppm. On the other hand, rest of the two bacterial antagonists could grow only up to 4000 ppm concentration cyclohexamide (Table 5). Very few literature reports exist on antibiotic sensitivity of thermophilic bacteria for comparison with our results. Imanaka et al. (1981) tested the thermophilic *Bacillus subtilis* strains isolated from hot springs and compost for resistance to antibiotics. They reported that bacteria were resistant to antibiotics like tetracycline (250 ppm) and streptomycin sulfate (1000 ppm). The antibiotic resistance of the thermophilic bacterial antagonists from present study indicates them as potential biocontrol candidates for safe integration with bactericides (antibiotics) commonly used in disease management.

Based on the morphological, biochemical and physiological characters, the bacterial isolates were identified upto species level as *B. licheniformis* (boiled cow milk), *B. stearothermophilus* (compost manure) and *B. stearothermophilus* (tomato rhizospheric soil). However, isolates from compost manure and tomato rhizospheric soil were different from each other in respect of their antagonistic potential (zone of inhibition produced) against test soil-borne fungal pathogens and therefore seems to be two different strains of same species *B. stearothermophilus*. The isolated bacterial strains were given names as BLbcm for *B. licheniformis* strain from boiled cow milk, BScm for *B. stearothermophilus* strain from compost manure and BStrs for *B. stearothermophilus* strain from tomato rhizosperhic soil.

## *In vitro* screening for antagonism against soil borne fungal plant pathogens

All the three thermophilic isolates produced strong inhibition zones against test pathogens in dual culture assay (Table 6, Figure 5). *B. licheniformis* has shown highest antagonism against four test soil borne fungal pathogens *viz*., *P. aphanidermatum, F. oxysporum* F. sp. *ciceri, R. bataticola* and *F. lycopersici* compared to *B. stearothermophilus* (compost manure) and *B. stearothermophilus* (tomato rhizospheric soil). However, the isolates of *B. stearothermophilus* were comparatively more effective against *R. solani* and *S. rolfsii* respectively than *B. licheniformis*. In *S. rolfsii* colour of mycelia/ hyphae approaching to antagonistic bacteria was

**Table 5.** Antibiotic sensitivity of thermophilic *Bacilli.*

| Antibiotic concentration (ppm) | Bacterial isolates from | | |
|---|---|---|---|
| | Boiled cow milk | Compost manure | Tomato rhizospehric soil |
| **Streptocyclin** | | | |
| 50 | ++++ | + | + |
| 100 | +++ | - | - |
| 250 | ++ | - | - |
| 500 | + | - | - |
| 1000 | - | - | - |
| **Streptomycin sulphate** | | | |
| 50 | ++++ | + | + |
| 100 | ++++ | - | - |
| 250 | ++++ | - | - |
| 500 | +++ | - | - |
| 1000 | ++ | - | - |
| **Bacterianashak** | | | |
| 50 | ++ | ++++ | ++++ |
| 100 | + | + | + |
| 250 | + | + | + |
| 500 | - | + | + |
| 1000 | - | - | + |
| **Cyclohexamide** | | | |
| 100 | ++++ | ++++ | ++++ |
| 500 | ++++ | ++++ | ++++ |
| 1000 | +++ | ++++ | ++++ |
| 3000 | +++ | +++ | +++ |
| 4000 | +++ | ++ | ++ |
| 6000 | ++ | - | - |
| 7000 | ++ | - | - |

**Table 6.** *In vitro* efficacy of three thermophilic antagonistic *Bacilli* against six major soil borne fungal pathogens.

| Test pathogen | Host plant | Inhibition zone (mm) | | | Inhibition (%) | | |
|---|---|---|---|---|---|---|---|
| | | *B. licheniformis* | B. *stearothermophilus* (compost manure) | *B. Stearothermophilus* (tomato rhizospheric soil) | *B. licheniformis* | *B. stearothermophilus* (compost manure) | *B. stearothermophilus* (tomato rhizospheric soil) |
| *P. aphanidermatum* | Brinjal | 63.00±1.80[b] | 41.33±1.80[b] | 38.33±1.80[a] | 70.00 | 45.93 | 42.59 |

**Table 6.** Contd.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *F. ciceri* | Chick pea | $34.17\pm0.29^{d}$ | $26.33\pm0.29^{c}$ | $28.67\pm0.29^{c}$ | 37.97 | 29.26 | 31.86 |
| *R. bataticola* | Chick pea | $67.67\pm2.25^{a}$ | $43.00\pm2.25^{b}$ | $34.83\pm2.25^{b}$ | 75.19 | 47.78 | 38.7 |
| *S. rolfsi* | Chick pea | $18.50\pm 0.25^{f}$ | $19.25\pm2.25^{d}$ | $23.00\pm0.25^{d}$ | 20.56 | 46.67 | 25.56 |
| *F. lycopersici* | Tomato | $30.83\pm1.15^{e}$ | $19.67\pm1.15^{d}$ | $23.33\pm1.15^{d}$ | 34.26 | 21.86 | 25.93 |
| *R. solani* | Tomato | $40.50\pm0.50^{c}$ | $51.67\pm0.50^{a}$ | $36.33\pm0.50^{ab}$ | 45.00 | 57.42 | 40.37 |
| SE (mean) | | 0.75 | 0.48 | 0.96 | | | |
| CD (@0.05%) | | 2.30 | 1.48 | 2.94 | | | |

The different superscript letters a-f within the column indicates the treatment is significantly different from each other.

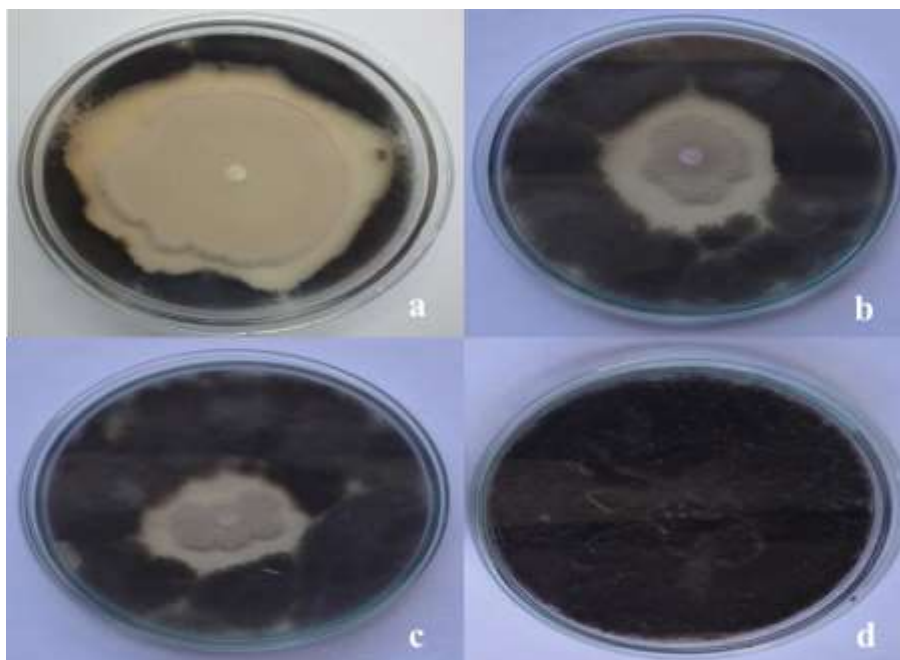



**Figure 5.** *In vitro* antagonism of thermophilic bacterial isolates against *Rhizoctonia solani* by inhibition zone technique. **a.** *B. licheniformis*; b. *B. stearothermophilus* from compost manure; c, *B. stearothermophilus* from tomato rhizospheric soil; **d,** control.

changed from white to brown which may due to antifungal compounds secreted by the bacterial antagonists. Similar results have been reported by earlier researchers. Landa et al. (2004) reported that approximately 32% of 74 bacterial isolates from the chickpea rhizosphere inhibited growth of

*F. oxysporum* f. sp. *ciceri* in dual cultures under *in vitro* studies. Foldes et al. (2000) reported that *B. subtilis,* strain IFS-01 isolated from rhizosphere of cereals produced clear inhibition zone against major phytopathogens *viz*., *F. oxysporum, Alternaria alternata, Botrytis cineria and Aspergillus niger*. Montealegre et al. (2003) reported that *B. subtilis* strain 639 and *B. lentimorbus* strain 640 isolated from rhizoplane and rhizosphere of healthy and diseased tomato plants produced halo zone against *R. solani* in dual culture assay. Garima et al., (2005) reported that the rhizobacteria, *Bacillus* sp. (GF23 and A555) significantly inhibited radial growth of soil borne fungal pathogens viz, *R. solani, S rolfsi, A. niger, A. flavus, Fusarium semitectum, F. udum, F. oxysporum f.* sp. *ciceri., F. moniliforme* and *F. oxysporum f.* sp. *lycopersici* by dual culture plate assay, thus showing a broad spectrum antagonism. Nakkeeran et al. (2006) reported that rhizobacteria *B. subtilis* strain BSCBE4 isolated from vegetable crops (tomato, brinjal and hot paper) showed the highest inhibitory effect on mycelial growth of *P. aphanidermatum* on PDA medium. Mehetre and Kale (2011) reported *in vitro* antagonism of thermophilic bacterium *B. licheniformis* against *P. aphanidermatum* causing chilli damping off. Sabet et al. (2013) reported that *Bacillus* sp. isolated from five commercial composts had antagonistic effects on soil borne fungal pathogens of cucumber *in vitro*. The treatment of antagonistic *Bacillus* strains B3, B5, B7, B9, and B11 suppressed the radial mycelial growth (24.4 to 57.8%) of *F. solani, P. ultimum*, *R. solani*, and *S. rolfsi* causing root rot in cucumber. Sharma et al. (2013) reported that *B. amyloliquefaciens* strain sks_bnj_1 isolated from diseased roots of soybean showed antagonism against *S. rolfsi, Sclerotinia* sp and *F. nivale*.

## Conclusions

The present study revealed strong antagonistic potential of three thermophilic bacteria against major soil-borne fungal plant pathogens. The biochemical and physiological characterisation of thermophilic bacterial isolates indicated their ability to tolerate high temperature, pH, salt and antibiotic concentrations. We propose the thermophilic bacterial isolates as the potential biocontrol candidates for management of soil-borne fungal plant pathogens, especially under stressed environments where other biocontrol agents fail. Further studies in relation to efficacy of thermophilic bacterial antagonists for control of soil borne plant pathogens under field conditions and development of suitable bioformulations for their efficient filed delivery are required before recommending them for biocontrol.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## REFERENCES

Akanbi TO, Kamaruzaman AL, Abu Bakar F, Abdul Hamid NS, Radu S, Abdul Manap MY, Saari N (2010). Highly thermostable extracellular lipase producing *Bacillus* strain isolated from a Malaysian hot spring and identified using 16S rRNA gene sequencing. International Food Research Journal 17:45-53.

Allen MB (1953). The thermophilic aerobic spore forming bacteria. Bacteriological Reviews 17:125-173.

Besson F, Peypoux F, Michel G, Delcambe L (1978). Identification of antibiotics of iturin group in various strains of *Bacillus subtilis*. The Journal of Antibiotics 31:284-288.

Biswas KK, Das ND (1999). Biological control of pigeon pea wilt caused by *Fusarium udum* with *Trichoderma* spp. Annals of Plant Protection Sciences 7:46-50.

Brannen PM, Backman PA (1994). Suppression of *Fusarium* wilt of cotton with *Bacillus subtilis* formulations. In: (Ryder, M.H., Stephens, P.M. and Bowen, G.D., editors) Improving plant productivity with rhizosphere bacteria. CSIRO, Division of Soils, Adelaide, Australia, pp. 83-85.

Cappuccino JG, Sherman N (1987). Microbiology: A laboratory manual. Dorling Kindersley Pvt. Ltd., Delhi, India. P 185.

Elnasser Z, Maraqua A, Owais W, Khraisat A (2007). Isolation and characterization of new thermophilic bacteria in Jordan. The Internet Journal of Microbiology 3:1-10.

Esikova TZ, Temirov YuV, Sokolov SL, Alakhov YuB (2002). Secondary antimicrobial metabolites produced by thermophilic *Bacillus* sp. strains VK2 and VK21. Applied Biochemistry and Microbiology 38:226-231.

Foldes T, Banhegyi I, Verga Z, Szigeti J (2000). Isolation of *Bacillus* strain from the rhizosphere of cereals and *in vitro* screening for antagonism against phytopathogenic, food-borne pathogenic and spoilage micro-organisms. Journal of Applied Microbiology 89:840-848.

Fujio Y, Kume S (1991). Isolation and identification of thermophilic bacteria from sewage sludge compost. Journal of Fermentation and Bioengineering 72:334-337.

Garima J, Bhat V, Anjaiah V (2005). Plant growth promoting activity of some rhizobacterial strains on tomato plants. Indian Phytopathology 58: 462-465.

Georgieva SS, McGrath SP, Hooper DJ, Chambers BS (2002). Nematode communities under stress: the long-term effects of heavy metals in soil treated with sewage sludge. Applied Soil Ecology 20: 27- 42.

Gulati HK, Chadha BS, Saini HS (2007). Production and characterization of thermostable alkaline phytase from *Bacillus laevolacticus* isolated from rhizosphere soil. Journal of Industrial Microbiology and Biotechnology 34:91-98.

Hoitink HAJ, Boehm MJ (1999). Biocontrol within the context of soil microbial communities: substrate-dependent phenomenon. Annual Review of Phytopathology 37:427-44.

Imanaka T, Fujii M, Aiba S (1981). Isolation and characterization of antibiotic resistance plasmids from thermophilic *Bacillus* sp. and construction of deleticon plasmids. Journal of Bacteriology 146:1091-1097.

Janstova B,Lukasova J, (2001). Heat resistance of *Bacillus* sp. spores isolated from Cow's milk and farm environment. Acta Veterinaria Brno 70:179-184.

Kim DS, Cook RJ, Weller DM (1997). *Bacillus* sp. L 324-92 for biological control of three root disease of wheat grown with reduced tillage.

Phytopathology 87:551-558.
Landa BB, Navas Cortes JA, Jimenez Diaz RM (2004). Influence of temperature on plant rhizobacterial interaction related to biocontrol potential for suppression of *Fusarium* wilt of chickpea. Plant Pathology 53:341-352.
Mehetre ST, Kale SP (2011). Comparative efficacy of thermophilic bacterium, *Bacillus licheniformis* (NR1005) and antagonistic fungi, *Trichoderma harzianum* to control *Pythium aphanidermatum* induced damping off in chilli (*Capsicum annuum* L.). Archives of Phytopathology and Plant Protection 44:1068-1074.
Miyatake F, Iwabuchi K (2005). Effect of high compost temperature on enzymatic activity and species diversity of culturable bacteria in cattle manure compost. Journal of Biotechnology 96:1821-1825.
Montealegre JR, Reyas R, Perez LM, Herrera R, Polyana S, Besoain X (2003). Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Journal of Biotechnology 6:115-127.
Morton DT Stroube NH (1955). Antagonistic and stimulatory effects of microorganism upon *Sclerotium rolfsi*. Phytopathology 45: 419-420.
Nakanishi H (1963). The effect of various pasteurization methods on survival of microorganisms in the raw milk supplies. Japanese Journal of Dairy and Food Science 12: A77.
Nakkeeran S, Kavitha K, Chandrasekari G, Renukadevi P, Fernando WGD (2006). Induction of plant defence compounds by *Pseudomonas chlororaphis* PA23 and *Bacillus* subtilis BSCBE4 in controlling damping off of hot pepper caused by *Pythium aphanidermatum.* Biocontrol Science and Technology 16:403-416.
Nunes de Souza A, Martins MLL (2001). Isolation, properties and kinetics of growth of a thermophilic *Bacillus.* Brazilian Journal of Microbiology 32: 271-275.
Oerke EC (2006). Crop losses to pests. Journal of Agricultural Science 144: 31-43.
Osburn RN, Milner JL, Oplinger ES, Smith RS, Handelsman J (1995). Effect of *Bacillus cereus* UW 85 on yield of soybean at two field sites in Wisconsin. Plant Disease 79:551-556.
Panda MK, Sahu MK, Tayung K (2013). Isolation and characterization of a thermophilic *Bacillus* sp. with protease activity isolated from hot spring of Tarabalo, Odisha, India. Iranian Journal of Microbiology 5:159-165.
Pathak AP, Rekadwad BN (2013). Isolation of thermophilic *Bacillus* sp. strain EF_ TYK1-5 and production of industrially important thermostable α amylase using suspended solid for fermentation. Journal of Scientific and Industrial Research 72:685-689.
Rao GV, Rupela OP, Rameshwar Rao V, Reddy YVR (2007). Role of biopesticides in crop protection: Present status and future prospectus. Indian Journal of Plant Protection 35:1-9.
Ronimus RS, Ruckert A, Morgan HW (2006). Survival of thermophilic spore-forming bacteria in a 90+ year old milk powder from Ernst Shackelton's Cape Royds Hut in Antarctica. Journal of Dairy Research 73:235-243.
Ruckert A, Ronimus RS, Morgan HW (2004). RAPD based survey of thermophilic *Bacilli* in milk powders from different countries. Food Microbiology 96:263-272.
Sabet, KK, Saber MM, El-Naggar MA, El-Mougy NS, El-Deeb HM, El-Shahawy IE (2013). Using commercial compost as control measures against cucumber root rot disease. The Journal of Mycology 13:1-13.
Santana MM, Portillo MC, Gonzalez JM, Clara MIE (2013). Characterization of new soil thermophilic bacteria potentially involved in soil fertilization. Journal of Plant Nutrition and Soil Science 176:47-56.
Seeley HW, Vandemark PJ (1970). *Microbes in Action-A laboratory manual of microbiology*. D.B. Taraporevala Sons and Company Pvt. Ltd., Mumbai. pp. 85-86.
Sharma SK, Aketi R, Johri BN (2013). Isolation and characterization of plant growth promoting *Bacillus amyloliquefaciens* strainsks_bnj_1 and its influence on rhizosphere soil properties and nutrition of soybean (*Glycine max* L. Merrill). Journal of Virology pp. 1-19.
Singh SK, Tripathi VR, Jain RK, Vikram S, Garg SK (2010). An antibiotic, heavy metal resistant and halotolerant *Bacillus cereus* SIU1 and its thermoalkaline protease. Microbial Cell Factories 9:59.
Snaeth PHA (1986). Section13: Endospore-forming gram positive rods and cocci. *In:* (Holt, J.G., editor) *Bergey's Manual of Systematic bacteriology*, Williams and Wilkins, Baltimore. pp. 1104-1207.
Van Zwieten L (2004). Impacts of pesticides on soil biota. In: (R. Lines Kelly, Editor) Soil biology in agriculture, Proceedings of a workshop on current research into soil biology in agriculture, Tamworth, NSW Department of Primary Industries. pp. 72-79.
Wightwick AM, Salzman SA, Reichman SM, Allinson G, Meinzies NW (2010). Inter-regional variability in environmental availability of fungicide derived copper in vineyard soils: An Australian case study. Journal of Agricultural and Food Chemistry 58:449-457
Xiao Y, Zeng GM, Yang ZH, Ma YH, Shi CHWJ, Xu ZY, Huang J Fan CZ (2011). Effects of continuous thermophilic composting (CTC) on bacterial community in the active composting process. Microbial Ecology 62:599-600.

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*Full Length Research Paper*

# Bacteriological quality of drinking water obtained from main sources, reservoirs and consumers' tap in Arba Minch town, Southern Ethiopia

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**The most common and wide spread health risk associated with drinking water is microbial contamination. The aim of this study is to assess microbial contamination of drinking water, starting from source to distribution systems. Water samples from the Arba Minch town of Southern Ethiopia were collected randomly from the main source before chlorination, reservoir after chlorination and from different points of distribution lines. Total coliforms, fecal coliforms and heterotrophic plate count (HPC) were determined from collected water samples. Coliforms were analyzed by using the most probable number (MPN) method. About 93.3% of collected water samples were contaminated with total coliforms and 16.7% of distributed tap water was contaminated with fecal coliforms. Most of the analyzed water samples had high number of viable bacteria or HPC (>5 log), and total coliforms. The HPC ranged from 1.9 log of bacteria in the chlorinated water in reservoir tank to 8.44 log in the source water before chlorination. Overall, the quality of drinking water suggests that the distribution lines are the most likely point of microbial contamination. Therefore, regular bacteriological monitoring and maintaining residual chlorine in distribution system is mandatory.**

**Key words:** Coliforms, drinking water, heterotrophic plate count, microbiological point of contamination.

## INTRODUCTION

Water contamination is the most common and widespread health risk in developing countries. About 663 million people in the world lacked contaminants-free drinking water sources according to UNICEF and WHO (2015)

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reports. According to the report, Ethiopia is one of the countries that have met the millennium development goal drinking water target (UNICEF and WHO, 2015). In the protection of drinking water, identification of the point of contamination is very important. The health and well-being of a population is directly affected by the coverage of water supply and sanitation.

The impact of poor water quality on the transmission of communicable diseases is well established (Usman et al., 2016; Schlipköter and flahault, 2010). The main problem associated with ingestion of water is microbial risk due to water contamination by human and animal feces. Some of the microorganisms found in water may cause diseases, thereby questioning its safety. Drinking such contaminated water or using it in food preparation may lead to new cases of infection. Many common and wide spread health risks have been found to be in association with drinking water in developing countries (Suthar et al., 2009). Furthermore, in developing countries, unsafe water, poor sanitation and hygiene problem have been reported to rank third among twenty leading risk factors for health burden (WHO, 2003).

Most of the population of Ethiopia does not have access to safe and reliable sanitation facilities. On top of these, majority of the households do not have sufficient understanding of hygienic practices regarding water and personal hygiene. As a result, over 75% of the health problems in Ethiopia are communicable diseases which resulted from having unsafe and inadequate water supply, and unhygienic waste management, particularly human excreta (WWAP, 2004). About three-quarter of health problems in children in the country is communicable disease arising from poor water supply and sanitation, most of which is associated with microbial contamination of drinking water (Kumie and Ali, 2005). In a study conducted in northern part of Ethiopia, about 46% of mortality in children of less than five year was due to diarrhea mainly related to unsafe drinking water (Asmassu et al., 2004).

The microbial quality of drinking water attracted great attention worldwide because of implied public health effects (Abdelrahman and Eltahir, 2011). The effective way of assessing water safety is by using water quality indicators microbes. The fecal indicator bacterium has been considered as biological indicators of drinking water for a long period of time (Enriquez et al., 2001). Detection of bacterial indictors in drinking water shows the presence of pathogenic organisms that are the source of water borne diseases which could be fatal. The point of contamination can vary depending on the water treatment condition and residual chlorine concentration in distribution lines (Amenu et al., 2013). Thus, water quality problem can be assessed by identifying indicator organism that shows existence of water contamination. Limited studies have been carried out to assess the point of microbial contamination of drinking water in southern part of Ethiopia. Therefore, this study was conducted to assess the microbial contamination of drinking water, starting from point source to distribution systems.

## MATERIALS AND METHODS

### Study design and setting

A cross sectional study was conducted to assess bacteriological quality of drinking water obtained from main sources, reservoirs and consumers' tap in Arba Minch town, Southern Ethiopia. Arba Minch is the capital town of the Gamo Gofa zone, located 500 km south of Addis Ababa in southern Ethiopia. It is situated in the great African rift valley with an elevation of 1285 m above sea level. The total area of the town is estimated to be about 1095 hectares. Its temperature is about 29°C and the average rain fall is 900 mm. The drinking water source of the Arba Minch town is from forty spring sources. The drinking water in the study area is treated by chlorination.

### Sample collection and sampling point

Samples were collected from fifteen different locations grouped into three types of water sources. Twelve tap water samples were collected from different sites of distribution system (customer taps), one from reservoir just after chlorination, two water samples from spring water as initial source before chlorination. The method of sample collection was according to WHO (2008) guidelines for sample collection for drinking water quality assessment. Samples were collected aseptically from each sampling site in sterile bottles with capacity of 250 mL and transported to the laboratory in ice box and the samples were analyzed within two hours of collection. For the chlorinated water samples, about 2.5 mL of 10 mg/mL sodium thiosulphate was added into each sampling bottle to stop the chlorination process during transportation. Microbiological analysis of water sample was done as soon as possible after collection to avoid unpredictable changes in the microbial population (WHO, 2008).

### Microbial analysis

Water samples were analyzed for heterotrophic plate count (HPC), total coliforms and fecal coliforms. The HPC which aims to count all microorganisms that is capable of growing on nutrient agar was performed by using serial diluted water sample from $10^{-1}$ to $10^{-6}$ dilution level. Then, 1 mL of each diluted water sample was inoculated on nutrient agar using pour plate method and incubated at 37°C for 24 h. The bacterial count was done and expressed as colony forming units (CFU) per mL (APHA, 1992).

Coliforms were enumerated by the most probable number (MPN) techniques using sets of three tubes inoculated with 10 mL of MacConkey broth (Oxoid®) with 1 mL of serial diluted at 1, 0.1 and 0.01 mL. The water analyses were carried out in two stages. The first test is presumptive test and it is performed by inoculating the three level diluted samples in tubes which contain the MacConkey broth and incubated at 37°C for 48h. After the period of incubation, the inoculums were examined for gas formation by inspecting displacement of liquid media by air in Durham's tubes. The first reading was taken after 24 h to record positive tubes, and negative tubes were incubated for another 24 h. Then, the formation of gas in the incubated culture media was considered as positive presumptive test.

A positive presumptive sample was further confirmed by confirmatory tests. In confirmatory test, 1 mL of inoculums in positive presumptive tubes were transferred to the three different

**Table 1.** Risk indicator microorganisms in different samples of pre and post chlorinated drinking water.

| Source of water sample | HPC CFU/mL (log/ml) | Total coli form MPN/100 mL (95% CI) | Fecal coli form MPN/100 mL |
|---|---|---|---|
| Sample from $Ss_1$ | $2.8 \times 10^7$ (7.45 log) | >2,400 | - |
| Sample from $Ss_2$ | $2.8 \times 10^8$ (8.45 log) | 1,100 (150, 4800) | - |
| Sample from Rs | $8.0 \times 10^1$ (1.9 log) | - | - |
| Sample from $Ds_1$ | $2.6 \times 10^7$ (7.4 log) | 460 (71, 2,400) | 3 |
| Sample from $Ds_2$ | $1.9 \times 10^8$ (8.29 log) | 240 (36, 1,300) | - |
| Sample from $Ds_3$ | $1.2 \times 10^8$ (8.1 log) | 150 (30, 440) | - |
| Sample from $Ds_4$ | $1.0 \times 10^8$ (8 log) | 460 (71, 2,400) | 15 |
| Sample from $Ds_5$ | $1.2 \times 10^5$ (5.1 log) | 240 (36, 1,300) | - |
| Sample from $Ds_6$ | $8.0 \times 10^6$ (6.9 log) | 20 (7, 89) | - |
| Sample from $Ds_7$ | $7.0 \times 10^5$ (5.8 log) | 9 (1, 36) | - |
| Sample from $Ds_8$ | $1.6 \times 10^4$ (4.2 log) | 7 (1, 23) | - |
| Sample from $Ds_9$ | $6.0 \times 10^4$ (4.77 log) | 14 (3, 37) | - |
| Sample from $Ds_{10}$ | $1.2 \times 10^7$ (7.1 log) | 93 (15, 380) | - |
| Sample from $Ds_{11}$ | $2.7 \times 10^8$ (8.4 log) | 460 (71, 2,400) | - |
| Sample from $Ds_{12}$ | $2.1 \times 10^8$ (8.3 log) | 240 (36, 1,300) | - |

Ss = Source of water sample; Rs = reservoir water sample 1; Ds = water sample taken after distribution. CFU: colony forming units; MPN: most probable number; CI: confidence interval.

dilution tubes of MacConkey broth and inoculated at 44.5°C for 48 h. Then gas produced samples inoculums in the respective tubes were recorded. Finally, the value obtained was interpreted using MPN table and expressed as MPN of coliform per 100 mL of the water sample.

The positive confirmatory test was further checked to complete the tests. In this case, the suspected organisms were inoculated on nutrient agar slant and tube of lactose broth. After 24 h at 37°C, the lactose broth was checked for the production of gas, and a Gram stain was performed from organisms grown on the nutrient agar (APHA, 1992). After the analysis which indicates the quality status of drinking water, the results were compared with the Ethiopia and WHO guideline values (WHO, 2004).

#### Quality control

Bacteriological quality of drinking water was processed according to standard operating procedures and laboratory safety rule was followed. Sample collecting bottles was sterilized before sample collection. To check sterility of prepared media, 5% of prepared batch of media were incubated overnight and checked for microbial growth in the media. Both positive and negative controls were inoculated together with test water sample.

#### Data analysis

Descriptive statistical methods were used to summarize data and the result of bacteriological analyses was compared with national and WHO guidelines for drinking water during interpretation.

## RESULTS

The HPC of collected water samples ranged from $8 \times 10^1$ to $2.80 \times 10^8$ CFU/mL water. The lowest HPC $8 \times 10^1$ CFU/mL was observed in the sample which was collected from the reservoir immediately after chlorination before entering distribution line, while the high number of HPC was detected from the spring water source before chlorination. Total coliforms and fecal coliforms determined for all the water samples are presented in Table 1.

Microbial analysis of source spring water sample taken from different points before chlorination showed heterotrophic plate count of $2.85 \times 10^7$ and $2.8 \times 10^8$ CFU/mL in both samples. The highest values (>2,400 MPN/100 mL) were detected for total coli forms in the spring water before chlorination and no fecal coliforms were detected after chlorination.

In order to assess the effectiveness of treatment, microbial properties of the drinking water was also assessed after chlorination before entering distribution lines. The number of cultivable microorganisms after chlorination was determined by heterotrophic plate count and about 2.9 log/mL organisms were detected. Unlike other water samples, the MPNs showed no total coliform and fecal coliform bacteria (Table 2). Microbial analysis was also performed on distributed tap water (chlorinated) taken from 12 different parts of the town (Figure 1). The heterotrophic plate count of tap water ranged from $1.6 \times 10^4$ to $2.8 \times 10^8$ CFU/mL with a mean of $7.8 \times 10^7$ CFU/mL. The total coliform counts ranged from 7.4 to 460 MPN/100 mL, while the fecal coliform counts were detected in two water samples which accounts 3 and 15 MPN/100 mL (Table 1).

All the spring source water and distributed tap water were positive for total coliforms. About 16.7% of distributed tap water samples also had fecal coliforms by most probable number analysis method. The reservoir water sample was free of both total coliforms and fecal coliforms (Table 2).

**Table 2.** Occurrence of indicator bacteria at source, from reservoir and tap water.

| Type of water sample | Type of indicator microorganisms | | Total number of collected samples |
|---|---|---|---|
| | Total coli form | Fecal coli form | |
| Source (spring water) | 2 | - | 2 |
| Reservoir after chlorination | - | - | 1 |
| Distributed tap water | 12 | 2 | 12 |
| Total | 14 | 2 | 15 |

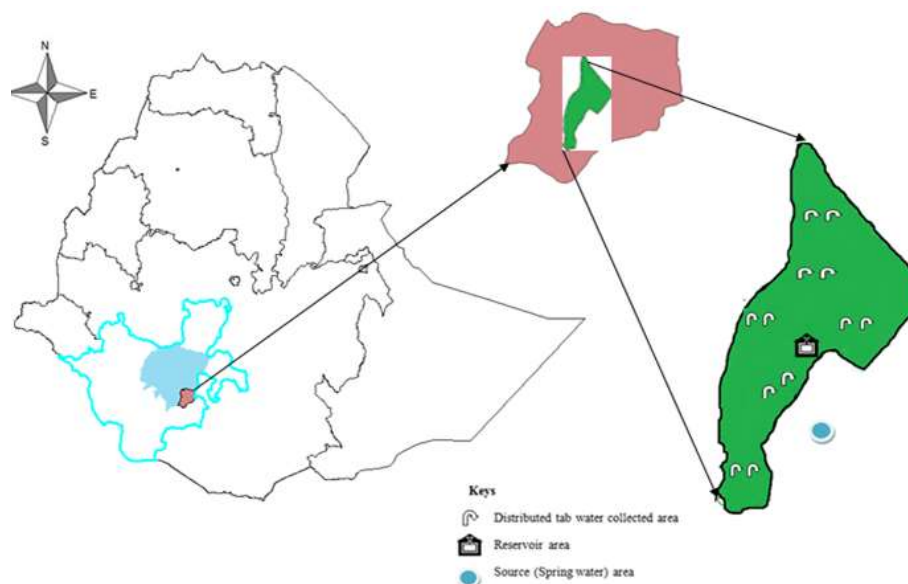


**Figure 1.** Map of the study sites, Arba Minch, Ethiopia.

Based on World Health Organization (1997) guidelines for drinking-water level of risk category for total coliform and fecal coliform, 16.7% of tap water sample fell within the low risk category, about 25% of tap water sample was classified as medium risk, and 58.3% of tap water was classified under high risk. The same as for total coliform level of risk for fecal coliform was determined. For fecal coliform indicator, about 83.4% of tap water samples had no risk, 8.3% was classified under low risk and similarly, 8.3% fell within the medium risk category (Figure 2).

## DISCUSSION

Heterotrophic plate count of non-chlorinated source water (spring water) was higher (>7 log) when compared with chlorinated reservoir water. This indicates that the action of chlorine in reducing high bacteria load of source water was very important. Although, the bacteria load of reservoir water was low, there were about $8 \times 10^1$ CFU/mL microorganisms. This may be due to the use of insufficient concentration of chlorine or due to the resistance organisms to effective concentration of chlorine disinfectant. According to a study conducted by Chouhan (2015), the number of HPC bacteria in drinking water ranged from <0.02 to $1 \times 10^4$ CFU/mL. Reductions of HPC levels from the raw source water to the finished water after treatment ranged from <1 log to 2 log for upland catchment water, and 1 log to 4 log for river derived water (WHO, 2003). Bacteriological quality changes may cause aesthetic problems involving taste and odor development, slime growths and colored water.

The microbial load of drinking water after leaving the treatment reservoir was high (> 4.2 log) as compared to its load before leaving the treatment reservoir (1.9 log). This result shows recontamination of drinking water in the distribution lines and the microbial load was higher than the amount of organism recommended by WHO (2004) standard. The growth of microorganisms after leaving the treatment site at the distribution network can be explained from different points of view. One possible reason for this high load of HPC in the distribution line is the insufficient residual chlorine level which is unable to inhibit the growth of microorganism. The other possible reason could be the distribution line damage and cross contamination with microorganisms. Similar findings were

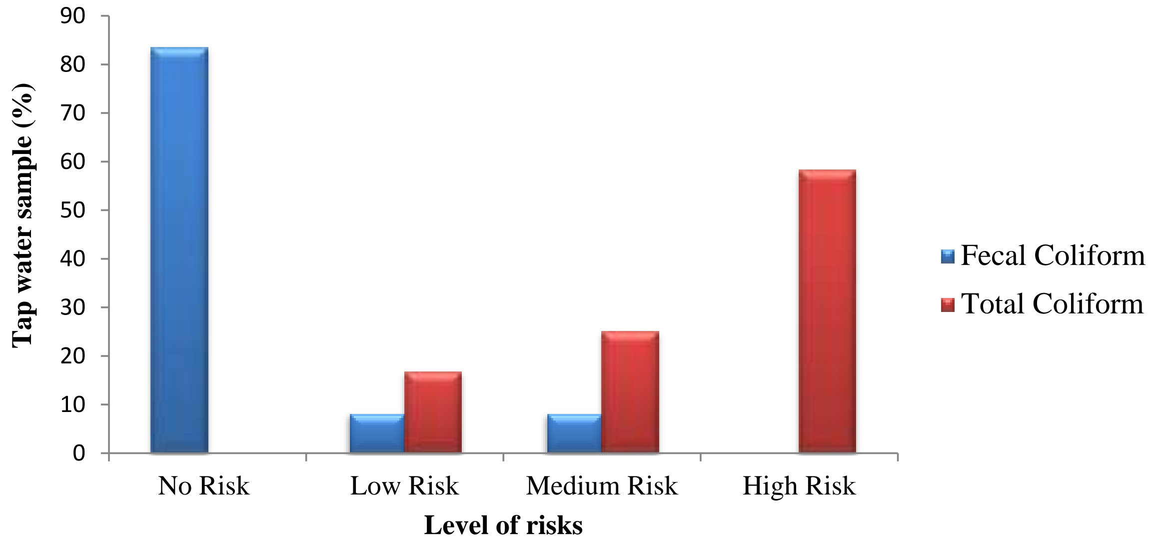


**Figure 2.** Level of the risk indicator organisms in chlorinated water collected from distribution lines.

exhibited in Ismailia canal water with HPC ranging from $1.0 \times 10^6$ to $4.13 \times 10^8$ CFU/mL (Abdo et al., 2010). In contrast, low HPC was observed in a study conducted on Gezira State drinking water (Elbakri, 2015). This might be due to lack of frequent repair of broken pipe line and less residual chlorine in the current study.

Highest number of total coliforms was recorded at the spring source water before treatment (>2,400 MPN/ml). Results of total and fecal coliforms revealed that the drinking water was unsafe according to national and WHO drinking water standards which states less than 10 coliform/mL of drinking water (UNEP, 1996). This high number of the coliforms may be due to soil and plant coliforms other than fecal origin. One of the possible reasons for these findings is contamination of water after leaving the reservoir through the damaged water pipe line. The other possible reason can be use of ineffective concentration of chlorine in the treatment reservoir. Furthermore, it can be due to low residual chlorine in the distribution system, contamination due to transient pressure drops leading to infiltration of ground water into water pipes, contamination due to incorrect cross connection with sewer lines, interconnection with toilet, pipe corrosion, pipe breakage and entrance of contaminants into the distribution system (Ailamaki et al., 2003).

Similar findings were obtained in a study done in Sri Lanka (Dissanayake, 2004), Bona District, Sidama Zone, Southern, Ethiopia (Berhanu and Hailu, 2015) and Bahir Dar city (Tabor et al., 2011). In this study, about 93.3% of analyzed water samples were positive to total coliform indicator bacteria and 16.7% of the samples were positive to fecal coliforms. Similar findings were observed in study conducted in Dire Dawa (Amenu et al., 2013). Unlike this study, all samples collected were positive to total coliforms. This difference may be due to differences in the sanitary facilities of the studied area (Amenu et al., 2013). In contrast to this study, a study conducted in Adama town, Oromia regional state of Ethiopia showed acceptable amount of coliforms according to WHO and national standards (Eliku and Sulaiman, 2015). The observed difference may be due to sufficient residual chlorine in the distribution line and appropriate water protection in Adama town.

Any coliform presence in drinking water is unacceptable even though their level of risk indication depend on the type of coliforms and number of coliforms present in a water sample. In drinking water, the presence of fecal coliforms should not be ignored as the basic assumption that pathogens would not be presented in drinking water, but this study shows the presence of fecal coli form. Since they are indicators of possible presence of waterborne pathogens, one can expect waterborne diseases in the study area. Due to the presence of indicator microorganism such as coliforms in drinking water, one can infer that there could also be water associated enteric or other pathogens such as *Salmonella* species, *Shigella* species, *Vibrio cholera*, etc. in the water. Properly constructed spring water may be free of fecal coliform bacteria. The presence of coliforms in spring water indicates leakage of surface water into the spring. It could also be due to poor construction or cracks in the spring casing.

The quality of drinking water is highly associated with the sanitary facility of the water catchment area. Therefore, the poor quality of drinking water observed may be as a result of poor sanitary condition of the area (WHO and UNICEF, 2014). On the other hand, in urban areas of Ethiopia, the availability of improved latrine, shared latrine, unimproved latrine and open defecation

accounts for about 27, 42, 23, and 8%, respectively (WHO and UNICEF, 2014). In 2015, 2.4 billion people in the world still had no access to improved sanitation facilities. The global population living in rural areas had seven out of ten people without improved sanitation facilities and nine out of ten people still practice open defecation (UNICEF and WHO, 2015).

## Conclusion

The amount of total coliforms and fecal coliforms detected in Arba Minch town drinking water were not in harmony with the standard set out by WHO for drinking water. The maximum level of HPC from all analyzed water sample at both spring water source and in distribution systems indicates that it is unsafe for drinking. The presence of high number of coliforms in the drinking water showed it is unsafe for consumption. Therefore, this should be considered by regulatory bodies as many diseases can be spread through fecal transmission. Regular monitoring of the distribution system for level of chlorine residue is mandatory. By considering these home water treatment mechanisms like granular-medium filters, home based physical and chemical disinfection is recommended.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

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## REFERENCES

Abdelrahman AA, Eltahir YM (2011). Bacteriological Quality of drinking water in Ngalas south Darfur, Sudan. Environmental Monitoring and Assessment 175(1-4):37-43.

Abdo MH, Sabae SZ, Haroon BM, Refaat BM, Mohammed AS (2010). Physico-chemical characteristics, microbial assessment and antibiotic susceptibility of pathogenic bacteria of Ismailia Canal Water, River Nile, Egypt. Journal of American Science 6(5):234-250.

Ailamaki A, Faloutsos C, Fischbeck PS, Small MJ, Vanbriesen J (2003). An environmental sensor network to determine drinking water quality and security. Sigmod Record 32(4):47-52.

Amenu D, Menkir S, Gobena T (2013). Assessing the bacteriological quality of drinking water from sources to household water samples of the rural communities of Dire Dawa Administrative Council, Eastern Ethiopia. Science, Technology and Arts Research Journal 2(3):126-133.

American Public Health Association (APHA) (1992). Companidum of method for the microbiological examination of food 3rd ed. Washington, DC, USA pp. 777-779.

Asmassu M, Wubshet M, Gelaw B (2004). A survey of bacteriological quality of drinking water in northern Gondar. Ethiopian Journal of Health Development 18(2):112-115.

Berhanu A, Hailu D (2015). Bacteriological and physicochemical quality of drinking water sources and household water handling practice among rural communities of Bona District, Sidama Zone-Zouthern, Ethiopia. Science Journal of Public Health 3(5):782-789.

Chouhan S (2015). Enumeration and identification of standard plate count bacteria in raw water supplies. IOSR Journal of Environmental Science, Toxicology and Food Technology 9(2):67-73.

Dissanayake SAMS, Dias SV, Perera MDC, Iddamalgoda IAVP (2004). Microbial Quality Assurance of Drinking Water Supplies through Surveillance. Environment Division, National Building Research Organization, Colombo, Sri Lanka. Water Professionals' Symposium.

Elbakri HK (2015). Evaluation of drinking water quality from surface and ground resource in Gezira State. PhD Thesis, University of Khartoum.

Eliku T, Sulaiman H (2015). Assessment of physico-chemical and Bacteriological quality of drinking water supply at sources and household in Adama town, Oromia Regional State, Ethiopia. African Journal of Environmental Science and Technology 9(5):413-419.

Enriquez C, Nwachuku N, Gerba CP (2001). Direct exposure to animal enteric pathogens. Reviews on Environmental Health 16(2):117-131.

Kumie A, Ali A (2005). An overview of environmental health status in Ethiopia with particular emphasis to its organization, drinking water and sanitation: A literature survey. Ethiopian Journal of Health Development 19(2):89-103.

Schlipköter U, Flahault A (2010). Communicable Diseases: Achievements and Challenges for Public Health. Public Health Reviews 32(1):90-119

Suthar S, Chhimpa V, Singh S (2009). Bacterial contamination in drinking water: a case study in rural areas of northern Rajasthan, India. Environmental Monitoring and Assessment 159(1-4):43-50.

Tabor M, Kibret M, Abera B (2011). Bacteriological and physicochemical quality of drinking water and hygiene-sanitation practices of the consumers in Bahir Dar City, Ethiopia. Ethiopian Journal of Health Sciences 21(1):19-26.

UNICEF, World Health Organization (2015). A 25 years Progress on Sanitation and Drinking Water. UNICEF and WHO. Geneva, Switzerland. Available on: https://www.unicef.pt/progressos-saneamento-agua-potavel/files/progress-on-sanitation-drinking-water2015.pdf

United Nations Environment Programme (UNEP) (1996). Water Quality Monitoring. A practical guide to the design and implementation of freshwater quality studies and monitoring programs. United nation Environment program and WHO; Chapman & Hall, London. http://www.who.int/water_sanitation_health/resourcesquality/waterqualmonitor.pdf

Usman MA, Gerber N, Braun JV (2016). The Impact of Drinking Water Quality and Sanitation Behavior on Child Health: Evidence from Rural Ethiopia, ZEF - Discussion Papers on Development Policy No. 221, Center for Development Research, Bonn, July 2016. P 45.

World Health Organization (1997) Guidelines for drinking-water quality, 2nd edition. Geneva: World Health Organization. http://www.who.int/water_sanitation_health/dwq/2edvol2p1.pdf.

World Health Organization (2003). Emerging issues in water and infectious disease. Geneva, WHO. Available on: http://www.who.int/water_sanitation_health/emerging/emerging.pdf

World Health Organization (2003). Heterotrophic Plate Counts and Drinking-water Safety. Edited by J. Bartram, J. Cotruvo, M. Exner, C. Fricker, A. Glasmacher. Published by IWA Publishing, London, UK. ISBN: 1843390256. http://www.who.int/water_sanitation_health/water-quality/guidelines/HPCintro.pdf?ua=1

World Health Organization (2004). Water, sanitation and hygiene links to health, facts and figures. Geneva, Switzerland. http://www.who.int/water_sanitation_health/en/factsfigures04.pdf .

World Health Organization (2008). Guidelines for Drinking Water Quality. 3rd Ed. WHO, Geneva, Switzerland. Available on: http://www.who.int/water_sanitation_health/publications/gdwq3rev/en/

World Health Organization and UNICEF (2014). Drinking water and sanitation; Progress on Drinking Water and Sanitation, UNICEF and World Health Organization. Geneva, Switzerland. Available on:

https://www.unicef.org/gambia/Progress_on_drinking_water_and_sanitation_2014_update.pdf
World Water Assessment Program (WWAP) (2004). National Water Development Report for Ethiopia. UN-WATER/WWAP/2006/7, Addis Ababa.

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# Phylogenetic diversity of prokaryotes on the snow-cover of Lewis glacier in Mount Kenya

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**The seasonal snowpack of the temperate glaciers are sources of diverse microbial inoculi. However, the microbial ecology of the tropical glacial surfaces is endangered, hence poses an extinction threat to some populations of some microbes due to rapid loss of the glacier mass. The aim of this study was to isolate and phylogenetically characterise the prokaryotes from the seasonal snow of Lewis glacier in Mt. Kenya. Snow samples were inoculated into Difco™ R2A Agar and BG-11 medium. Genomic DNA of seventeen representative axenic isolates was extracted using the mixture of MP FastDNA soil kit and the 16S rDNA gene region partially sequenced. The 16S rDNA gene sequences were blastn analyzed against the Genbank database and phylogenetic analysis was performed using MEGA 6 software. Phylogenetic analysis grouped the isolates into three phyla: Firmicutes, Proteobacteria and Actinobacteria. Isolates were affiliated with the genera *Bacillus* (53%), *Stenotrophomonas* (23.4%), *Cryobacterium* (5.9%), *Paenibacillus* (5.9%), *Subtercola* (5.9%) and *Arthrobacter* (5.9%). The results confirm that the seasonal tropical snowpack of Lewis glacier is dominated by the general terrestrial prokaryotes and a few glacier and snow specialist species.**

**Key words:** Seasonal snowpack, tropical glacier, prokaryotes, 16S rDNA.

## INTRODUCTION

Cold-adapted regions, ice cores from Polar Regions and glaciers from mid-latitude and high-latitude mountains are known to harbour diverse and active microbial community structures (Palmisano and Sullivan, 1983; Grebmeier and Barry, 1991; Skidmore et al., 2005). Ice cores are important for dating past microbiological diversity such as the bacteria, fungi and algae that are present in ice cores from polar ice sheets and mountain glaciers (Christner et al., 2003; Miteva et al., 2009).

Snow algae and yeast cells can accumulate and multiply on glacier surfaces from temperate regions, in accumulation areas because of the availability of meltwater, which is essential for their growth and nutrient cycling (Uetake et al., 2011). Cold-adapted yeasts have been isolated from supraglacial and subglacial ice in Svalbard (Butinar et al., 2007), Austrian glacier ice (Margesin et al., 2007), Italian subglacial meltwater (Buzzini et al., 2012), supraglacial and subglacial ice and

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meltwater from the Italian Alps (Turchetti et al., 2007), glacial and subglacial waters from northwest Patagonia (Brizzio et al., 2007), and an Antarctic deep ice core (Amato et al., 2009).

Temperate glaciers are characterised with the seasonal snow cover that harbours phytoflagellates, mostly the micro-algae (Anesio and Laybourn-Parry, 2012). Snow-packs are good sources of microbial inoculi (Hallbeck, 2009), nutrients (Schmidt and Lipson, 2004), and water (Barnett et al., 2005) that cascades through the drainage tills forming the melt water at the subglacial ecosystems. The availability of the inorganic nitrogen and phosphate compounds (Uetake et al., 2010) on glacier environments are controlled by the phototrophic activity of the snow algae and the snow-pack Cyanobacteria (Hodson et al., 2008). 16S rRNA gene sequencing has also revealed the availability of *Proteobacteria, Firmicutes* and *Actinobacteria* that are able to degrade organic compounds such as the propionate, acetate and formate that are available on the Arctic snow (Hodson et al., 2008).

The diversity and ecology of the prokaryotes in the snowpack from the rapidly disappearing tropical African glaciers, Mt. Kilimanjaro, 5895 m, Mt. Rwenzori, 5109 m and Mt. Kenya, 5199 m is not yet studied. In this study, the phylogenetic diversity of prokaryotes in the snowpack from Lewis glacier in Mt. Kenya was determined using culture based approach. Lewis glacier on Mt. Kenya is the smallest (0.4 $km^2$ in 1993) in Africa (Kaser, 1999) and biggest glacier on Mt. Kenya (Lewis Glacier) that is rapidly shrinking (Prinz et al., 2011). The study revealed that the snowpack of the rapidly disappearing Lewis glacier is dominated by the Firmicutes, Genus *Bacillus.* This is the first report to explain the availability of the prokaryotes from the psychrophilic ecosystems in the African continent.

## MATERIALS AND METHODS

### Sample collection and processing

Snow samples were collected in September, 2016 from Lewis glacier (latitude 0° 9′ 30″S - 0° 9′ 15″S, longitude 37° 18′ 45″E - 37° 19′ 0″E, Figure 1) in Mt. Kenya, Nyeri county. Samples were collected from 5 sites (ST1, ST2, ST3, ST4 and ST5). At each site, 5 samples were collected at a different of 0.1 × 0.1 m areas and stored in sterile 50 ml falcon tubes for cell counts and isolation. All samples were collected using pre-cleaned stainless steel scoops and spoons. In the field, samples were kept cold around 0°C in large stainless steel vacuum flasks with glacial ice samples before they were transported to Jomo Kenyatta University of Agriculture and Technology Laboratory for analyses.

### Isolation and purification of isolates

Sterile syringe was used to inoculate 200 µl of melt snow into plates containing appropriate heterotrophic bacterial media, Difco™ R2A Agar with the following composition: yeast extract (0.5 g), No. 3 proteose peptone (0.5 g), casamino acids (0.5 g), dextrose (0.5 g), soluble starch (0.5 g), sodium pyruvate (0.3 g), dipotassium phosphate (0.3 g), magnesium sulfate (0.05 g), and agar (15.0 g). While for the autotrophic prokaryotes, BG-11 was used with the following composition: $Na_2MG$ EDTA (0.5 g), ferric ammonium citrate (0.6 g), citric acid.$1H_2O$ (3.6 g), $MgSO_4.7H_2O$ (7.5 g), $K_2HPO_4.3H_2O$ (4.0 g), $H_3BO_3$ (2.86 g), $MnCl_2.4H_2O$ (1.81 g), $ZnSO_4.7H_2O$ (0.222 g), $CuSO_4.5H_2O$ (0.079 g), $COCl_2.6H_2O$ (0.050 g), and $NaMoO_4.2H_2O$ (0.391 g). Each of the components were mixed and brought up to a 1 L volume of distilled water at pH 7.2. Inoculants were incubated at 25°C. Growth rates were monitored daily for a week while sub-culturing until axenic cultures were obtained.

### Genomic DNA (gDNA) extraction and 16S rRNA gene amplification

Pure isolates were used for the extraction of the gDNA. A colony was picked from plates of fresh cultured isolates using a sterile wire-loop and directly added to a mixture of MP FastDNA soil kit according to the manufacturer's protocol. Purified DNA was quantified photometrically (NanoDrop; Thermo Fisher Scientific, Germany) and used as a template for amplification of 16S rRNA genes using the general bacterial primer pair 27F [5'-AGAGTTTGATCCTGGCTCAG-3'] and 1492R [5'-GGTTACCTTGTTACGACTT-3'] (Lane, 1991). PCR amplification was performed using TaKaRa Ex Taq DNA polymerase (Takara, Shiga,Japan). For each PCR, 1 µl (25 ng/µl) of the template was mixed with TaKaRa Ex Taq™ HS (5 units/µl) according to the manufacturer's protocol. The PCR conditions were as described by Mackenzie et al. (2007) except the final extension which was at 72°C for 8 min. PCR product size was checked using a 1% agarose gel stained with ethidium bromide. The amplicons were gel purified using Macherey-Nagel NucleoSpin extract II kit as per the manufacturer's protocol and eluted in 30 µl of TE Buffer (5 mM, pH 8.0).

### DNA sequencing and phylogenetic analysis

Polymerase chain reaction (PCR) products were sequenced with a 3130xl Genetic Analyzer (Applied Biosys-tems, California) at the National Institute of Polar Research (NIPR, Japan). Sequences of the isolates were manually edited in chromas and checked for presence of artifacts or chimeric structures using the Mallard software (Ashelford et al., 2006). A search for similar sequences using BLASTN program was performed, and sequence alignment was performed using the CLUSTAL Omega program (http://www.clustal.org). A neighbor-joining tree of the aligned sequences was constructed using MEGA V6 (Tamura et al., 2011). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). To obtain support values for the branches, bootstrapping (Felsenstein, 1985) was conducted with 1000 replicates. All sites, including gaps in the sequence alignment, were excluded pair-wise in the phylogenetic analysis. Using the resultant neighbor-joining tree, each isolate was assigned to the proper taxonomic group. The taxonomic assignment was confirmed at a 90% confidence level using the naïve Bayesian rRNA classifier on the RDP website (Cole et al., 2013). All sequences were deposited in GenBank nucleotide database with the accession numbers MH329929 to MH329948.

## RESULTS

### Affiliation of 16S rRNA gene sequences of the isolates

A total of 17 representative isolates from snowpack were

**Table 1.** Taxonomic affiliation and percentage sequence similarities of bacterial isolates with closest relatives from the Genbank database.

| Sample ID | Accession No. | Closest taxonomic affiliation | Isolation source | ID (%) |
|---|---|---|---|---|
| Lewis Bac 1 | MH329929 | *Bacillus subtilis* strain NB-01 (HM214542) | Forest floor | 100 |
| Lewis Bac 4 | MH329932 | *Bacillus subtilis* strain K21 (JN587510) | Fermented soy food | 100 |
| Lewis Bac 18 | MH329945 | *Bacillus tequilencis* strain JO-17 (MF321840) | Saline desert soil | 100 |
| Lewis Bac 2 | MH329930 | *Cryobacterium* species (AB872307) | Cryoconite sediment | 100 |
| Lewis Bac 3 | MH329931 | *Bacillus safensis* strain CF4 (KY085985) | Corn rhizosphere | 100 |
| Lewis Bac 7 | MH329935 | *Bacillus pumilus* ZB13 (EF491624) | NA | 96 |
| Lewis Bac 16 | MH329943 | *Bacillus safensis* strain U41 (CP015610) | Lake Untersee | 96 |
| Lewis Bac 5 | MH329933 | *Stenotrophomonas maltophilia* (LT906480) | Mouth | 100 |
| Lewis Bac 6 | MH329934 | *Stenotrophomonas maltophilia* strain Nc 15MA-2(KP296212) | Mouth | 100 |
| Lewis Bac 13 | MH329940 | *Stenotrophomonas maltophilia* strain 2681 (CP008838) | NA | 100 |
| Lewis Bac 21 | MH329948 | *Stenotrophomonas maltophilia* strain 2681 (CP008838) | NA | 100 |
| Lewis Bac 8 | MH329936 | *Bacillus niabensis* strain G3-1-20 (KC494318) | Soil | 100 |
| Lewis Bac 10 | MH329937 | *Paenibacillus taichungensis* strain 043(JN975184) | Plant root rhizosphere | 100 |
| Lewis Bac 14 | MH329941 | *Bacillus thuringiensis* strain 043 (KY323329) | Baltic sea | 100 |
| Lewis Bac 15 | MH329942 | *Bacillus horikoshii* strain 20a (CP020880) | Sediment | 99 |
| Lewis Bac 19 | MH329946 | *Lysinimonas* species (MG934620) | Glacier | 100 |
| Lewis Bac 20 | MH329947 | *Arthrobacter agilis* strain L77 (AY131225) | Psychrotolerant | 100 |

selected from the axenic colonies for phylogenetic analyses. The isolates (prefixed as Lewis Bac with their accession numbers in parenthesis) in the inferred phylogenetic tree were phylogenetically diverse and affiliated with known members from different phyla including *Firmicutes, Proteobacteria,* and *Actinobacteria* (Table 1 and Figure 1).

Comparison of the newly isolated 16S rRNA gene sequences to known sequences in the Genbank database using Blastn analysis indicated sequences similarities of >96% with known sequences (Table 1). Most of the isolates (53%) were closely affiliated with members of the genus *Bacillus* with >96 sequence identity. Four isolates (23.5%; Lewis Bac 5, 6, 13 and 21) had 100% sequence identities with known members of the genus *Stenotrophomonas*. The other four isolates (Lewis Bac 2, 10, 19 and 20) had 100% sequence identities with known members of the genera *Cryobacterium, Paenibacillus, Subtercola* and *Arthrobacter,* respectively. Three isolates (17.6%; Lewis Bac 2, 19 and 20) had 100% identities to known glacier and polar zone genera *Cryobacterium, Subtercola/Agreia* and *Arthrobacter,* respectively. 76.5% of the isolates had >96% identity to known members of the terrestrial and aquatic ecosystems while the other 5.9% of the isolates had 100% identity to known members of the human gastrointestinal tract, genus *Stenotrophomonas.*

The phylogenetic tree formed two major clusters described by the possible sources of the isolates. Snow or the psychrotolerants together formed a single sub-cluster supported with a bootstrap value of 100% (Figure 1). The terrestrial and aquatic isolates were also clustered together with sub-clusters describing sub-species and strains of various genera (Figure 1). Lewis Bac 5, 6, 13 and 21 were clustered together with the known genus *Stenotrophomonas.*

## DISCUSSION

Snowpack of Lewis glacier is a rich psychrophilic ecosystem that is conducive for the microbial inoculi. The snow cover of Lewis is, however, experiencing the downwind bio-aerosol input from the surrounding tall rocky points, moraine, terrestrial, human activity and savannah forests from Laikipia county. In this study, the isolates were partially identified to be closely related to the human gut pathogens, terrestrial and psychrophilic microbial ecology. The molecular analyses of 16S rRNA gene sequences revealed three phyla, *Firmucutes*, *Proteobacteria,* and *Actinobacteria* as the colonies on the Lewis glacier snowpack. The presence of the three phyla *Firmicutes, Proteobacteria,* and *Actinobacteria* on the snowpack would be an indication of active degradation of organic compounds from the glacier surface as compared to their known features on glaciers elsewhere (Hodson et al., 2008). The occurrence of these phyla is also characterized with the balance of nutrient cycling between the atmosphere and glacier surface. Elsewhere, Jones (1999), Hodson et al. (2005) and Hodson et al. (2008) have shown that nitrogen cycling is important to the melting polar glaciers, but it would be much faster in the tropical glaciers, which are actively exposed to photosynthetic and heterotrophic processes in addition to

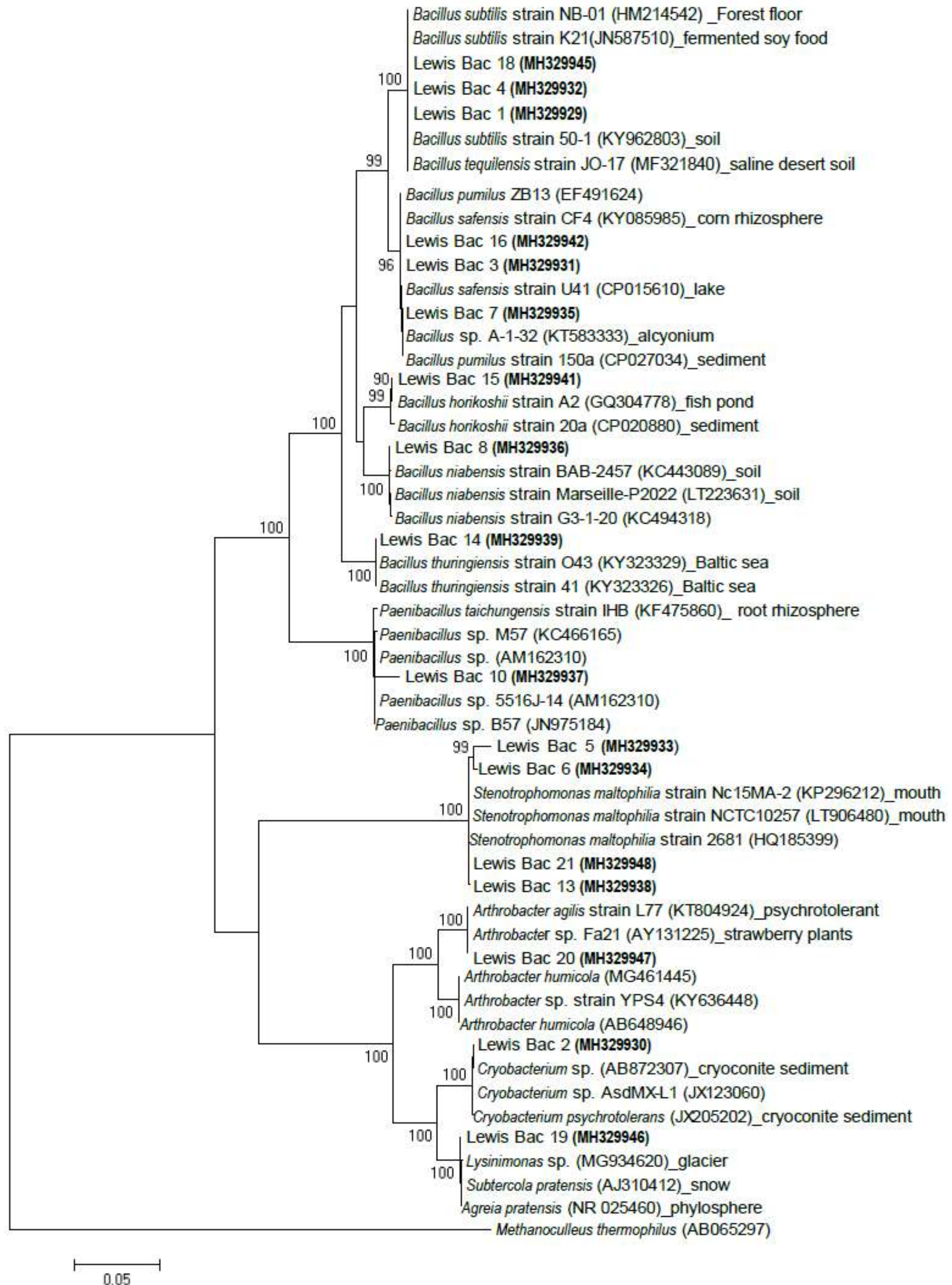


**Figure 1.** Evolutionary relationships between partial 16S rRNA gene sequences of the isolates and some selected known bacterial species. *Methanoculleus thermophiles* (AB065297) was used to root the tree.

other biogeochemical processes due to available litter and bio-aerosols influxes. However, the biogeochemical role of snowpack biota in nutrient cycling is not well understood.

The succession process of the rapidly disappearing Lewis glacier also contributes to the litter input on the snowpack. The genus *Bacillus* was the most dominant group on the glacier snowpack with a percentage of 53%. This can be explained by the strategic location of the glacier site to the windward and leeward sides where it experiences the upwind predominant weather systems from Indian ocean and downwind influxes from the savannah zones on Nanyuki, respectively. The exposure of the glacier surface to continuous leeward dust flow makes it a recipient settling zone for the contaminating terrestrial bio-aerosols. Most of the analyzed isolates (12; 53%) are soil and plant root rhizosphere symbionts, while 4 (23.5%) are closely related to human gut pathogens. Probably, the symbionts would be easily blown by the downwind from the savannah zones and oceanic upwind from the glacier foreland, which is colonized by the vascular plants (Schutte et al., 2009; Davey et al., 2015) onto the snowpack that acts as a settling point for litters and bio-aerosols. Moreover, soil inoculants would arise from the glacier terminal moraine that is re-colonized by the snow generalist phyla. The phylum *Proteobacteria* is a snow generalized colony, which is known to play a significant role in the rock mineralization at the glacier foreland (Yoshitake et al., 2010) that extends to the moraine ecosystem.

Isolates Lewis Bac 5, 6, 13 and 21 were identical to known members of the human pathogen (genus *Stenotrophomonas*) indicating that human activities on the glacier and also the surrounding influxes are possible sources. *Stenotrophomonas maltophilia* is ubiquitous in aqueous environments, soil, and plants (Berg et al., 1996, 1999), which are great sources to possible contaminant on the Lewis glacier snowpack. They are also known to be useful in wide range of biotechnology applications (Bhattacharya et al., 2007; Ryan et al., 2008). However, their occurrence in cryophilic environment is a rear finding that is reported for the first time from the tropical glacier.

In this study, only three snow specialist species (17.6%; isolates Lewis Bac 2, 19 and 20) were partially identified out of the 17 isolates. These three isolates were closely related to known groups of glacier specialist species of the genera *Cryobacterium, Subtercola/Agreia* and *Arthrobacter* (Figure 1) that have only been published from the mid-latitude to polar regions (Hodson et al., 2008). The genus *Cryobacterium* is psychrophilic (Suzuki et al., 1997; Zhang et al., 2007). *Cryobacterium psychrotolerans* is aerobic bacterium isolated from the China No. 1 glacier. They grow well between 4 and 27°C with an optimum growth at 20 to 22°C (Zhang et al., 2007). These conditions can as well be inhabited in the tropical Lewis glacier, which seems to have a constant temperature conditions. *Arthrobacter agilis* is a psychrotrophic bacterium, which occurs in lake water and Antarctic sea ice (Bowman et al., 1997; Deming, 2002). It produces dimethylhexadecylamine, plant growth promoting enzymes and cold active hydrolytic enzymes (Nadeem et al., 2013). These elements would be useful for the species competence, degradation of bioactive elements and cold shock stability within the tropical glacier ecosystem. Due to the seasonal snow fall on Lewis glacier and rapid melt of the englacial and subglacial zones of Lewis glacier (Hastenrath, 1983, 2006; Prinz et al., 2011), these psychrophilic prokaryotes are endangered and can be easily lost through the glacial surface run off and melt water tillage to the glacier bed and melting points.

Generally, the stability of microbial interactions on the snowpack is complexed with the climate change, duration of snow melt and rainfall (Hodson et al., 2008). However, the snowpack layer of Lewis glacier is relatively thin and has shorter melting durations, most likely due to the location of the glacier closer to the equator with a constant temperature. It means that the phototrophs are not affected by the snow flurries and their interactions with the snow heterotrophs have impact on the glacial interface microbial community structures, which may facilitate the rate of biogeochemical processes leading to rapid glacier melt. Lewis glacier snowpack is not colonized by the phototrophic prokaryotes as usual to other glaciers worldwide (Stanier and Bazine, 1977; Harding et al., 2011). This might be due to the seasonal snow fall characteristic of the Lewis snow cover, which is coupled with the influx of the bio-aerosols. The phototrophic prokaryotes might be colonizing the submerged cryoconite holes on the Lewis glacier surface, but not on the snowpack.

## Conclusion

The findings in this study suggest that the snowpack of the tropical Lewis glacier is colonized by diverse prokaryotes, including those of clinical and biotechnological significance. Only a few of the isolates were cryophilic that might be endangered by the rapid loss of the glacier. A number of isolates from the snowpack are, however, the general colonizers that are blown in by the downward and upward wind from the surrounding terrestrial, moraine, savannah ecosystems and the predominant oceanic weather system.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

Amato P, Doyle S, Christner BC (2009). Macromolecular synthesis by yeasts under frozen conditions. Environmental Microbiology 11:589-596.

Anesio AM, Laybourn-Parry J (2012). Glaciers and ice sheets as a biome. Trends in Ecology and Evolution (Amst.) 27:219-225.

Barnett TP, Adam JC, Lettenmaier DP (2005). Potential impacts of a warming climate on water availability in snow-dominated regions.

Nature 438:303.
Berg G, Marten P, Ballin G (1996). *Stenotrophomonas maltophilia* in the rhizosphere of oilseed rape-occurrence, characterization and interaction with phytopathogenic fungi. Microbiological Research 151:19-27.
Berg G, Roskot N, Smalla K (1999). Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. Journal of Clinical Microbiology 37:3594-3600.
Bhattacharya D, Nagpure A, Gupta RK (2007). Bacterial chitinases: properties and potential. Critical Reviews in Biotechnology *27*:21-28.
Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA (1997). Diversity and association of psychrophilic bacteria in Antarctic sea ice. Applied and Environmental Microbiology 63:3068-3078.
Brizzio S, Turchetti B, De Garcia V, Libkind D, Buzzini P, Van Broock M (2007). Extracellular enzymatic activities of basidiomycetous yeasts isolated from glacial and subglacial waters of northwest Patagonia (Argentina). Canadian Journal of Microbiology 53:519-525.
Butinar L, Spencer-Martins I, Gunde-Cimerman N (2007). Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. Antonie Van Leeuwenhoek 91:277-289.
Buzzini P, Branda E, Goretti M, Turchetti B (2012). Psychrophilic yeasts from worldwide glacial habitats: diversity, adaptation strategies and biotechnological potential. FEMS Microbiology Ecology 82:217-241.
Christner BC, Kvitko BH, Reeve JN (2003). Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. Extremophiles 7:177-183.
Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Tiedje JM (2013). Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Research 42:D633-D642.
Davey M, Blaalid R, Vik U, Carlsen T, Kauserud H, Eidesen PB (2015). Primary succession of *Bistorta vivipara* (L.) Delabre (Polygonaceae) root-associated fungi mirrors plant succession in two glacial chronosequences. Environmental Microbiology 17:2777-2790.
Deming JW (2002). Psychrophiles and polar regions. Current Opinion in Microbiology 5:301-309.
Felsenstein J (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.
Grebmeier JM, Barry JP (1991). The influence of oceanographic processes on pelagic-benthic coupling in polar regions: a benthic perspective. Journal of Marine Systems 2:495-518.
Hallbeck L (2009). Microbial processes in glaciers and permafrost. A literature study on microbiology affecting groundwater at ice sheet melting, SKB Rapport R-09-37. Svensk Kärnbränslehantering AB, Swedish Nuclear Fuel and Waste Management Co., Stockholm, Sweden.
http://www.iaea.org/inis/collection/NCLCollectionStore/_Public/41/033/41033788.pdf
Harding T, Jungblut AD, Lovejoy C, Vincent WF (2011). Microbes in high arctic snow and implications for the cold biosphere. Applied and Environmental Microbiology 77:3234-3243.
Hastenrath S (1983). Net balance, surface lowering, and ice-flow pattern in the interior of Lewis Glacier, Mount Kenya, Kenya. Journal of Glaciology 29:392-402.
Hastenrath S (2006). Diagnosing the decaying glaciers of equatorial East Africa. Meteorologische Zeitschrift 15:265-271.
Hodson A, Anesio AM, Tranter M, Fountain A, Osborn M, Priscu J, Sattler B (2008). Glacial ecosystems. Ecological Monographs 78:41-67.
Hodson A, Kohler J, Brinkhaus M, Wynn P (2005). Multi-year water and surface energy budget of a high-latitude polythermal glacier: evidence for overwinter water storage in a dynamic subglacial reservoir. Annals of Glaciology 42:42-46.
Jones HG, Pomeroy JW, Davies TD, Tranter M, Marsh P (1999). $CO_2$ in Arctic snow cover: landscape form, in-pack gas concentration gradients, and the implications for estimation of gaseous fluxes. Hydrological Processes 13:2977-2989.
Kaser G (1999). A review of the modern fluctuations of tropical glaciers. Glob. Planet Change 22:93-103.
Margesin R, Hämmerle M, Tscherko D (2007). Microbial activity and community composition during bioremediation of diesel-oil-contaminated soil: effects of hydrocarbon concentration, fertilizers, and incubation time. Microbial Ecology 53:259-269.
Miteva V, Teacher C, Sowers T, Brenchley J (2009). Comparison of the microbial diversity at different depths of the GISP2 Greenland ice core in relationship to deposition climates. Environmental Microbiology 11:640-656.
Nadeem SM, Naveed M, Zahir ZA, Asghar HN (2013). Plant–microbe interactions for sustainable agriculture: fundamentals and recent advances. In Plant Microbe Symbiosis: Fundamentals and Advances (pp. 51-103). Springer India.
Palmisano AC, Sullivan CW (1983). Sea ice microbial communities (SIMCO). Polar Biology 2:171-177.
Prinz R, Fischer A, Nicholson L, Kaser G (2011). Seventy-six years of mean mass balance rates derived from recent and re-evaluated ice volume measurements on tropical Lewis Glacier, Mount Kenya. Geophysical Research Letters 38:20.
Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008). Bacterial endophytes: Recent developments and applications. FEMS Microbiology Letters 278:1-9.
Schmidt S K, Lipson DA (2004). Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. Plant Soil 259:1-7.
Schütte UM, Abdo Z, Bent SJ, Williams CJ, Schneider GM, Solheim B, Forney LJ (2009). Bacterial succession in a glacier foreland of the High Arctic. International Society for Microbial Ecology Journal 3:1258.
Skidmore M, Anderson SP, Sharp M, Foght J, Lanoil BD (2005). Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. Applied and Environmental Microbiology 71:6986-6997.
Stanier RY, Bazine GC (1977). Phototrophic prokaryotes: the cyanobacteria. Annual Review of Microbiology 31:225-274.
Suzuki KI, Sasaki J, Uramoto M, Nakase T, Komagata K (1997). *Cryobacterium psychrophilum* gen. nov., sp. nov., nom. rev., comb. nov., an obligately psychrophilic actinomycete to accommodate "*Curtobacterium psychrophilum*" Inoue and Komagata 1976. International Journal of Systematic and Evolutionary Microbiology 47:474-478.
Tamura K, Nei M, Kumar S (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America 101:11030-11035.
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28:2731-2739.
Turchetti B, Buzzini P, Goretti M, Branda E, Diolaiuti G, D'Agata C, Vaughan-Martini A (2007). Psychrophilic yeasts in glacial environments of alpine glaciers. FEMS Microbial Ecology 63:73-83.
Uetake J, Kohshima S, Nakazawa F, Takeuchi N, Fujita K, Miyake T, Narita H, Aizen V, Nakawo, M. (2011). Evidence for propagation of cold-adapted yeast in an ice core from a Siberian Altai glacier. Journal of Geophysical Research Biogeosciences 116.
Uetake J, Naganuma T, Hebsgaard MB, Kanda H, Kohshima S (2010). Communities of algae and *Cyanobacteria* on glaciers in west Greenland. Polar Science 4:71-80.
Yoshitake S, Uchida M, Koizumi H, Kanda H, Nakatsubo T (2010). Production of biological soil crusts in the early stage of primary succession on a High Arctic glacier foreland. New Phytologist 186:451-460.
Zhang DC, Wang HX, Cui HL, Yang Y, Liu HC, Dong XZ, Zhou PJ (2007). *Cryobacterium psychrotolerans* sp. nov., a novel psychrotolerant bacterium isolated from the China No. 1 glacier. International Journal of Systematic and Evolutionary Microbiology 57:866-869.

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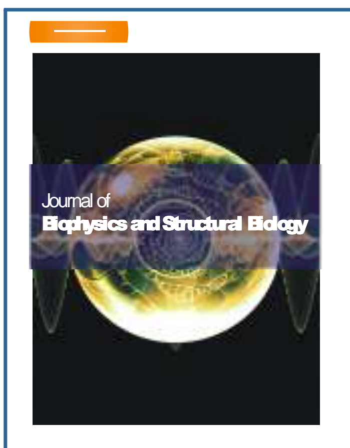

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