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Review

The biology and potential biotechnological applications of *Bacillus safensis*

Agbaje Lateef[1]*, Isiaka Adedayo Adelere[1,2] & Evariste Bosco Gueguim-Kana[3]

[1]*Microbiology Unit, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria; e-mail: agbaje72@yahoo.com; alateef@lautech.edu.ng*
[2]*Department of Microbiology, Federal University of Technology, Minna, Nigeria*
[3]*School of Life Science, Department of Microbiology, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa*

Abstract: *Bacillus safensis* colonizes a wide range of habitats, many of which are stringent for the survival of some microorganisms. Its survival in extreme environments relies on its unique physiological and genotypic characteristics. It was originally identified as a recalcitrant contaminant in a spacecraft-assembly facility (SAF) at the Jet Propulsion Laboratory, USA, from which it derived its specific epithet, *safensis*. The bacterium belongs to the *Bacillus pumilus* group, and is closely related to *Bacillus pumilus*, *Bacillus altitudinis*, *Bacillus xiamenensis* and *Bacillus invictae*. At times, *B. safensis* has been erroneously identified as *B. pumilus*, especially when extensive molecular analyses and some mass spectroscopic methods, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), are not considered. *B. safensis* possesses some plant growth-promoting traits and also has promising biotechnological applications due to its ability to produce various industrial enzymes and industrially applicable secondary metabolites. It may be regarded as a safe industrial microorganism because its pathogenicity has never been evidenced. This review attempts to chronicles the biology of *B. safensis* and its exploit as a potential industrially important bacterium. The ecology, physiology, genetics, and biotechnological applications of *B. safensis* are hereby presented in this review. This represents the first compendium of information on its attributes and applications that may be useful in opening a new vista of research on the bacterium.

Key words: *Bacillus safensis*; keratinase; dehairing; destaining; biocatalysis; nanoparticles; plant growth promotion.

Abbreviations: MALDI-TOF-MS matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SAF, spacecraft-assembly facility.

Introduction

Bacillus safensis is a Gram-positive, mesophilic, spore-forming, aerobic and chemo-heterotrophic bacterium. It is a rod-shaped and motile bacterium with high tolerance for salt, heavy metals, and ultraviolet and gamma radiations (Satomi et al. 2006; Raja & Omine 2012; Kothari et al. 2013). It has been described as a model microorganism for the identification of inactivated bacterial endospores using a novel technique that was based on fluorescence microscopy and propidium monoazide (Probst et al. 2012), which is important in differentiating between living and dead bacterial endospores to assess the level of decontamination in areas such as food industries and spacecraft programmes. *B. safensis* was first isolated as a contaminant from spacecraft-assembly facility (SAF) at the Jet Propulsion Laboratory, USA, from which it derived its specific epithet (Satomi et al. 2006). It colonizes wide range of habitats, some of which are mostly severe for other organisms to thrive (extreme environments). The habitats include spacecraft and associated environments, saline desert, industrial effluents, oil polluted sites, rhizosphere, insect guts, plant body, human and animal excreta, soil and others. Along with *Bacillus pumilus*, it is one of the most widespread species of the *B. pumilus* group (Branquinho et al. 2014a).

Strains of *B. safensis* capable of producing industrial enzymes, such as amylase (Kothari et al. 2013), cellulase (Khianngam et al. 2014), protease (Berrada et al. 2012; Kothari et al. 2013), lipase (Kumar et al. 2014), xylanase (Chi et al. 2012), chitinase (Berrada et al. 2012), inulinase (Singh et al. 2013; Singh & Singh 2014), keratinase (Lateef et al. 2015a) and β-galactosidase (Nath et al. 2012b, 2013) have been reported. It is still applicable as a plant growth-promoting bacterium (Kothari et al. 2013), bio-control agents (Berrada et al. 2012), probiotic (Nath et al. 2012a) and bioremediating organisms (Motesharezadeh & Savaghebi-Firoozabadi 2011). These unique abilities of *B. safensis* make it an ideal candidate for various biotechnological applications (Table 1). In this paper, we re-

* Corresponding author

Table 1. Sources, growth conditions and biotechnological applications of some strains of *B. safensis*.

Strain	GenBank Acc. No.	Source of isolation	Growth conditions			Biotechnological applications	References
			Temp (°C)	pH	NaCl (%)		
FO-36b^T	AF234854	Spacecraft and associated environment	30–37	5.6	0-10	–	Satomi et al. (2006)
VK	AUPF00000000	Rhizosphere	–	4–8	14	Plant growth promotion	Kothari et al. (2013)
JUCHE 1	–	Sweet meat whey	37	7.0	–	Probiotic	Nath et al. (2012b)
MS11	JF836885	Desert	30	7.0	15	Bioremediation and biogeochemical cycling of arsenic	Raja & Omine (2012)
URX303	–	Organic compost	39	7.0	–	Production of amylase	Pascon et al. (2011)
MX47	JN578480	Decaying wood	37	–	–	Production of xylanase	Chi et al. (2012)
B9	–	Magura cave	40	–	–	Production of various industrial enzymes	Tomova et al. (2013)
Rhf-2	–	Fruit	37	–	–	Production of various industrial enzymes	Khianngam et al. (2013)
BBN7	–	Hydrocarbon contaminated soil	23	–	–	Bioremediation	Mathe et al. (2012)
PJ1-24S	–	Oil palm meal	37	7.0	3–5	Production of cellulase	Khianngam et al. (2014)
YACN14	–	Pond water	27	–	3	Biocontrol	Zheng et al. (2012)
CB4	JN120810	Medicinal plant	28	–	–	Biocontrol	Sun et al. (2013)
LMA4	–	Rhizosphere	28	–	–	Plant growth promotion	Kavamura et al. (2013)
W10	–	Rhizosphere	–	–	–	Plant growth promotion	Chakraborty et al. (2013)
AS-08	–	Rhizosphere	25–42	6–9	0.5–12.0	Production of endoinulinase	Singh et al. (2013)
1–1	–	Soil	37	7	–	Production of lipase	Reza et al. (2014)
Eleven strains	–	Salt mines	35–37	7	1–25	Numerous applications	Roohi et al. (2014)
AS7	AB720127	Palm oil contaminated site	30	–	–	Production of biosurfactant	Saisa-Ard et al. (2013)
Three strains	–	Marine sediment	–	–	–	Production of biosurfactant	Porob et al. (2013)
E7	–	Fermented food	37	–	–	Food fermentation	Ahaotu et al. (2013)
DSM 19292	–	Fermented seeds	37	–	–	Food fermentation	Agbobatinkpo et al. (2013)
Two strains	JQ624775, JQ624766	Human cerumen	37	–	–	Degradation of cholesterol	Gerchman et al.(2012)
SF147, SF188	–	Soil	30	–	–	Production of carotenoid	Khaneja et al. (2010)
SMh-5	–	Fermented plant	35	–	–	Production of flavonoid	Kwon & Ha (2012)
MaI11-9	AB666457	Activated sludge sample	23	7.0	–	Biocontrol	Yang et al. (2012)
FO-036b	–	Lead and zinc mine	–	–	–	Plant growth promotion and bioremediation	Motesharezadeh & Savaghebi-Firoozabadi (2011)
PG1	–	Fermented cow dung	28	–	–	Plant growth promotion and biocontrol	Radha & Rao (2014)
–	–	Tannery effluent	32	–	–	Effluent treatment	Suganya et al. (2013)
–	–	Insect gut	28	–	–	–	Gupta et al. (2014)
DVL-43	KC156603	Soil	37	7.0	–	Enzymatic esterification	Kumar et al. (2014)
NBPP93	–	Soil	–	–	–	h Plant growth promotion and production of xylanase	Yadav et al. (2011)
LAU 13	KJ461434	Feather dump site	37	–	–	Keratinase, feather degradation, dehairing, destaining, and green synthesis of silver nanoparticles	Lateef et al. (2015a,b)

view the biology and potential biotechnological applications of *B. safensis*. To the best of our knowledge, this report represents the first attempt to summarize the ecology, physiology, genetics, and biotechnological applications of *B. safensis* in a single compendium.

Ecology

B. safensis colonizes a wide range of habitats that include terrestrial and marine environments (Liu et al 2013; Branquinho et al. 2014b). It is a robust bacterium that possesses a very remarkable physiologi-

cal attributes, which enable it to survive extreme and diverse environmental conditions known to be severe to other microorganisms (Satomi et al., 2006; Kothari et al. 2013). Its isolation from spacecraft and associated environments that harbour a very low biomass because of stringent maintenance (La Duc et al. 2003) could be an indication of its recalcitrance to decontamination techniques and the ability to survive extreme conditions. It has also been isolated as endophytic and plant growth-promoting rhizobacterium (Bibi et al. 2012; Chakraborty et al. 2013; Kavamura et al. 2013; Khianngam et al. 2013; Kothari et al. 2013; Souza et al. 2013; Edelman & Yin 2014), as organic matter decomposer (Chi et al. 2012) and as contaminants on fresco surfaces (GenBank accession No. KC429638), soil (Reza et al. 2014), chilli and egg plant (Achari & Ramesh 2014), marine zooplanktons (GenBank accession No. KF835731), tilapia (GenBank accession No. KC469708), insect gut (Gupta et al. 2014), bottled water cooler (Farhadkhani et al. 2014), oil palm meal (Khianngam et al. 2014), floral nectar (Fridman et al. 2012), casein whey (Nath et al. 2012a,b), cave (Tomova et al. 2013), animal excreta (Radha & Rao 2014), human cerumen (Gerchman et al. 2012), organic compost (Pascon et al. 2011), herbal medicinal products (Onyambu et al. 2013) and canned products (Velezmoro et al. 2012). Several authors have also reported the isolation of *B. safensis* strains from desert environments (Raja & Omine 2012; Kothari et al. 2013). Deserts are extreme environments for microorganisms and characterized with conditions like low moisture, high soil salinity, nutrient deficiency, high ultraviolet radiation and temperature, and strong winds. Its isolation from salt mines, hypersaline environment, heavy metals and hydrocarbon contaminated sites has also been reported (Berrada et al. 2012; Mathe et al. 2012; Roohi et al. 2014). The presence of *B. safensis* had been confirmed in several industrial wastes, like electroplating wastewater, tannery effluent treatment plant and in the activated sludge sample collected from a sewage treatment plant (Yang et al. 2012; Mekuto et al. 2013; Suganya et al. 2013). Similarly, fermented products have been reported to contain strains of *B. safensis* (Kwon & Ha 2012; Agbobatinkpo et al. 2013; Ahaotu et al. 2013; Kpikpi et al. 2014).

Physiology and phenotypic characteristics

B. safensis is a Gram-positive, aerobic, mesophilic, chemo-heterotrophic and motile spore forming bacterium. Their cells are rod shaped with 1.0–1.2 μm in length and 0.5–0.7 μm in diameter (Satomi et al. 2006). Moreover, it is a halophilic bacterium that tolerates high concentration of salt (1–25%) (Raja & Omine 2012; Kothari et al. 2013; Roohi et al. 2014). *B. safensis* also evidenced growth capacity at a pH range of 4.0–9.0 and within a temperature range of 10–50 ℃, with optimum growth at pH 7 and 37 ℃ (Satomi et al. 2006; Kothari et al. 2013; Singh et al. 2013; Roohi et al. 2014). The growth of *B. safensis* on tryptic soy agar produced whitish, round, undulate, dull, non-luminescent colonies with irregular margins (Satomi et al. 2006; Roohi et al. 2014). Moreover, Nath et al. (2012b) comparing the growth of a *B. safensis* β-galactosidase-producing strain in lactose broth and in a modified de-Mann Rogosa Sharpe medium, evidenced a highest growth in the latter. The growth of *B. safensis* has also been reported to be inhibited by Tween 40, 60 and 80 (Satomi et al. 2006; Singh et al. 2013).

Several authors have carried out extensive biochemical tests on strains of *B. safensis* and confirmed their reactions in varying degrees to some of the tests. *B. safensis* showed positive reaction to oxidase, catalase, alkaline phosphatase, β-galactosidase, β-glucosidase, esterase and Voges-Proskauer tests, but negative to H_2S, indole, amylase, leucinearyl amidase, cystinearyl amidase, valinearyl amidase, trypsin, tryptophan deaminase, α-galactosidase, phenylalanine deaminase, arginine dihydrolase, lysine decarboxylase, DNase, agarase, lecithinase, urease, nitrate reduction and ornithine decarboxylase (Satomi et al. 2006). In another study, Singh et al. (2013) reported *B. safensis* AS-08 that showed positive reaction to casein, gelatin, and esculin hydrolysis but negative to citrate and Voges-Proskauer tests. However, Raja & Omine (2012) gave a report of another strain that was negative to casein and starch hydrolysis but positive to citrate test.

Satomi et al. (2006) in their study further described *B. safensis* as a bacterium capable of acid production from D-glucose, D-xylose, fructose, mannose, galactose, inositol, mannitol, methyl α-D-mannopyranoside, glycerol, ribose, methyl α-D-glucopyranoside, L-arabinose, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, sucrose, trehalose, D-turanose and D-tagatose, but not from D-arabinose, adonitol, erythritol, methyl β-D-xylopyranoside, sorbose, rhamnose, dulcitol, sorbitol, melezitose, raffinose, inulin, starch, glycogen, xylitol, D-xylose, D-fucose, L-fucose, D-arabitol, L-arabitol, L-xylose, gluconate, 2-ketogluconate or 5-ketogluconate, L-arabitol, L-xylose, and gluconate. Similar biochemical characteristics of *B. safensis* have also been reported (Raja & Omine 2012; Singh et al. 2013; Reza et al. 2014).

The survival of organisms in any extreme environment relies on their efficient physiological and genotypic characteristics. Tirumalai et al. (2013) compared the genome of an ultraviolet- and a hydrogen-resistant strains, *B. pumilus* SAFR-032 and *B. safensis* FO-36b^T, respectively, with a closely related species *B. pumilus* ATCC 7061^T not capable of producing resistant spores, with the aim of identifying the genomic features responsible for this character. It was concluded that the association of several genes were responsible for the evidenced resistance exhibited by the spores of *B. safensis* FO-36b^T. Moreover, *B. safensis* MS11 (GenBank accession No. JF836885) isolated from Mongolia desert (Raja & Omine 2012) exhibited high resistance to arsenic and boron and also to heavy metals, such as cadmium, chromium, copper, nickel, lead, and zinc. Additionally, Mathe et al. (2012) described another

heavy-metal tolerant *B. safensis* BBN7 isolated from hydrocarbon and heavy metals contaminated sites. The strain showed resistance to antibiotics, like amoxicillin, penicillin, cephalosporin, ceftazidime, cefotaxime, and aminocumarin. Similarly, a strain of *B. safensis* isolated from casein whey was reported to exhibit strong resistance to streptomycin, bacitracin, erythromycin and chloramphenicol. Its growth was enhanced by common prebiotics, such as galactooligosaccharide, Indian garlic, onion and a natural antioxidant, Indian chilly (Nath et al. 2012a). *B. safensis* can also be referred to as a drought tolerant bacterium as its isolation from the rhizosphere of a Brazilian drought tolerant cactus was reported by Kavamura et al. (2013). These diverse physiological characteristics of *B. safensis* make it potentially applicable in broad range of biotechnological applications.

Genetics and taxonomy

The whole circular chromosome of *B. safensis* had been sequenced and the DNA G+C contents were determined to be 41.0–46.1 % (Satomi et al. 2006; Kothari et al. 2013). The genomic DNA of *B. safensis* harbours genes encoding enzymes for its plant growth-promoting potential, 3,928 proteins and 73 tRNA (Kothari et al. 2013). Taxonomically, the bacterium belongs to the *B. pumilus* group, having *B. pumilus*, *Bacillus altitudinis*, *Bacillus xiamenensis* and *Bacillus invictae* as the most closely related species (Branquinho et al. 2014c). These species are phenotypically and genotypically alike and can be distinguished using DNA homology studies. Some isolates of *B. safensis* have erroneously been identified as *B. pumilus*, especially when extensive molecular analyses were not considered. Classical molecular analyses like phylogenetic analyses of 16S rRNA as well as *gyrB* and *pyrE* gene sequences, repetitive-PCR fingerprinting, and DNA-DNA hybridization have been used to vividly distinguish *B. safensis* from *B. pumilus* (Satomi et al. 2006; Liu et al. 2014). Similarly, Agbobatinkpo et al. (2013) reported that *B. safensis* shared 90.2% *gyrA* sequence similarity with *B. pumilus* which is almost the same with the result (91.2% *gyrB* sequence similarity) obtained by Satomi et al. (2006). These results are suggesting that *gyrA* and *gyrB* could be used as marker molecules for the identification of *B. safensis*.

In addition, Liu et al. (2013) reported that both the *gyrB* and *pyrE* genes can be used as molecular marker to distinguish the closely related strains of *B. pumilus* and *B. safensis* multilocus sequence analysis of seven housekeeping genes. However, the authors concluded that the multilocus sequence analysis method might not be adequate enough to completely differentiate *B. safensis* from *B. pumilus* as the housekeeping genes used occupy only 0.1∼0.2% of the genome. Therefore, other fingerprinting methods, like randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism PCR (AFLP), Rep-PCR and genome sequence analyses have been proposed as alternative techniques that would differentiate them in more details (Liu et al. 2013).

However, some recent studies have shown that alternative techniques, e.g., matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) combined with chemometrics and Fourier transform infra red spectroscopy (FTIR) with attenuated total reflectance can be used to accurately and rapidly identify strains of *B. safensis* (Branquinho et al. 2014b,c). The studies established MALDI-TOF-MS and Fourier transform infra red spectroscopy technique as a fast and reliable method to identify bacterial strains, which can be easily implemented in the laboratory. It has been further suggested that ribosomal and spore proteins constituted most of the *B. pumilus* and *B. safensis* biomarkers, whose fingerprinting by MALDI-TOF-MS and other MS-based techniques can be used for rapid and accurate identification of *B. safensis* (Branquinho et al. 2014b).

Biotechnological applications

Several strains of *B. safensis* have been reported to possess attributes and also produced metabolites that can be exploited industrially for diverse biotechnological applications.

Endophytic plant growth promotion

Plants and microorganisms have been known to co-exist in nature, in which case the association can be either beneficial or harmful to the plant on which the microorganisms depend for carbon sources (Weisskopf 2013). They benefit plants in terms of growth promotion and health stimulation. Some endophytic and rhizobacteria secrete metabolites that stimulate plant growth and as well confer resistance against phytopathogens. *B. safensis* has proven to be a useful tool in sustainable agriculture as a biocontrol agent. There exist several reports of *B. safensis* as endophytic and plant growth-promoting rhizobacterium capable of producing growth-promoting traits, such as phosphate solubilization, production of siderophore, indole-3-acetic acid and 1-aminocyclopropane-1-carboxylate deaminase (Yadav et al. 2011; Chakraborty et al. 2013; Kavamura et al. 2013). *B. safensis* was reported to enhance growth in six varieties of wheat plant and also improved their abilities to withstand water stress (Chakraborty et al. 2013). Similarly, the involvement of *B. safensis* had been reported in the fermentation of cow dung used in organic farming to promote plant growth (Radha & Rao 2014).

Pathogens, particularly fungi, have been implicated in tremendous losses in agriculture world-wide (Strange & Scott 2005; Fisher et al. 2012) thereby limiting agricultural sustainability. Previously, diseases caused by pathogens have been controlled by chemical-based drugs but the strategy has some limitations. They do not adequately control plant infections, interfere with ecosystem and may also promote resistant pathogens (Zheng et al. 2012). Biological prevention and treatment of diseases have been proven to

be a new, promising way for disease control in recent years, because this method mainly utilizes antagonistic effects among microbes to inhibit the growth of pathogenic organisms, by using harmless microbes to suppress the growth of pathogenic organisms (Zheng et al. 2012). Plant growth-promoting rhizobacteria have been studied extensively for promoting plant growth and for inducing resistance to pathogens. *B. safensis* is capable of producing antifungal, antibacterial and antiviral effects in various crops (Berrada et al. 2012; Weisskopf 2013; Sun et al. 2014). It has been reported (Zheng et al. 2012) to produce strong antagonistic effect against two bacterial pathogens, *Pseudoalteromonas* sp. and *Pseudoalteromonas tetraodonis* associated with skin ulceration and peristome tumescence of *Apostichopus japonicus* (sea cucumber). *B. safensis* CB4 (GenBank accession No. JN120810) isolated from a Chinese medicinal plant produced inhibitory activity against pathogenic fungi and bacteria (Sun et al. 2013). The severity of phytophthora blight caused by *Phytophthora capsici* on squash has been significantly reduced by treatment with plant growth-promoting rhizobacteria strains; *B. safensis* and *Lysinibacillus boronitolerans* under greenhouse conditions (Zhang et al. 2010). In addition, *B. safensis* has also been used as a biocontrol agent against Oomycetous plant pathogens (Bibi et al. 2012), causative agent of tomato grey mould (Berrada et al. 2012) and harmful cyanobacterium *Microcystis aeruginosa* (Yang et al. 2012).

Source of industrial enzymes

Strains of *B. safensis* can veritably serve as sources of reputable industrial enzymes with a wide range of applications. *B. safensis* is a source of industrial important enzymes such as amylase, lipase, protease, cellulase, protease, chitinase, inulinase, keratinase and β-galactosidase. *B. safensis* has been reported as a good lipase-producing bacterium (Reza et al. 2014). Similarly, a strain of *B. safensis* produced an organic solvent stable lipase, which was used in an esterification reaction for the production of ethyl laurate (flavour ester) (Kumar et al. 2014). This quality indicates that the strain of *B. safensis* could be a promising tool in the production of lipase, which may have applications in flavours, fine chemicals, detergents, cosmetics and pharmaceutical industries.

Fructooligosaccharides, which are enzymatically produced using sucrose and inulin, are popular prebiotics because of their good health-promoting properties with a lot of food and pharmaceutical applications (Lateef et al. 2007a,b, 2008, 2012; Lateef & Gueguim-Kana 2012; Ganaie et al. 2014). Singh et al. (2013) described *B. safensis* as a potent source of endoinulinase, an enzyme that produces fructooligosaccharides from the hydrolysis of inulin. The production of the enzyme by the bacterium was optimized using response surface methodology (Singh & Singh 2014), thereby demonstrating the feasibility of the industrial production of the enzyme. Moreover, the synthesis of β-galactosidase, an important enzyme in dairy industry has been reported by a strain of *B. safensis* isolated from casein whey (Nath et al. 2012b, 2013). In addition, Chi et al. (2012) have isolated numerous xylanase-producing strains from decaying wood, out of which *B. safensis* MX47 (JN578480) produced the highest xylanase activity. Xylanase is a microbial enzyme that is indispensable in textiles, paper and pulp industries. Also, the cell wall degrading protease and β-1,3-glucanase have been produced by an antagonistic endophytic strain of *B. safensis* (Bibi et al. 2012). Strains of *B. safensis* that produced substantial protease, amylase and cellulase activities have been reported (Pascon et al. 2011; Tomova et al. 2013; Khianngam et al. 2014).

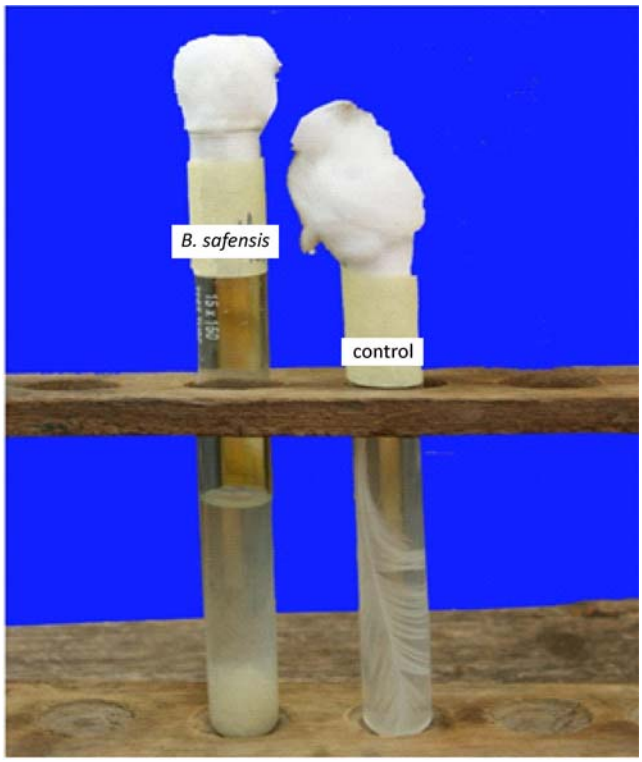

Fig. 1. Complete degradation of whole feather by *B. safensis* LAU 13.

Recently, in our laboratory a novel feather-degrading *B. safensis* LAU 13 (GenBank accession No. KJ461434) isolated from a feather dump site produced a very significant keratinase activity (Lateef et al. 2015a). The enzyme proved to be a promising tool for biotechnological applications as it displayed a very remarkable dehairing and destaining abilities (Figs 1–3). It also has potential nanobiotechnological application, as the keratinase through green synthesis produced quality spherical silver nanoparticles (Fig. 4) of 5–30 nm in size with good antibacterial activities against strains of *E. coli* (Lateef et al. 2015b). Our work represents the first reference to *B. safensis* as producer of keratinase for various biotechnological applications, including the green synthesis of silver nanoparticles.

B. safensis can still be considered a relevant organism in molecular biology since it has the potential for the production of restriction endonucleases (Espinoza-

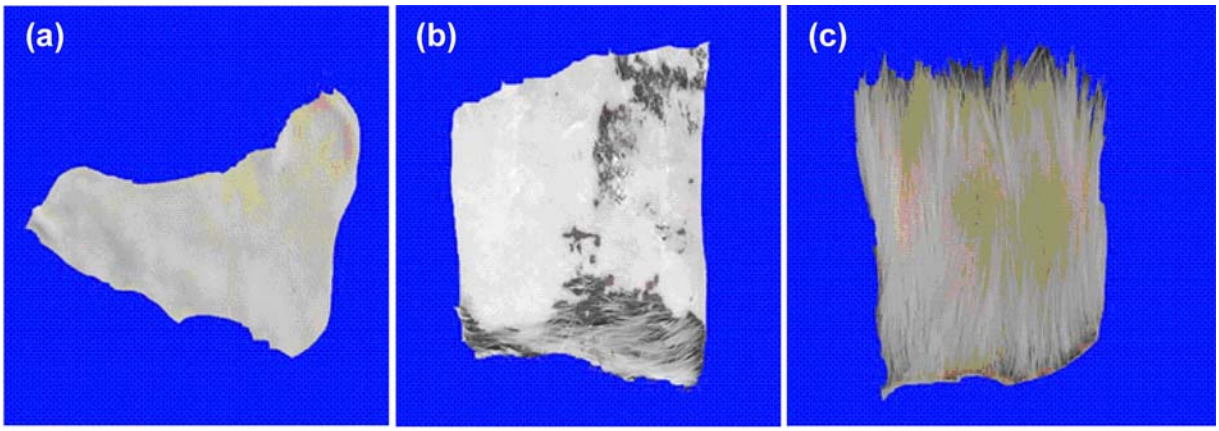

Fig. 2. Complete dehairing of goat skin by crude keratinase from wild strain of *B. safensis* LAU 13 (a), and incomplete dehairing by sodium sulphide and lime (b); control (c).

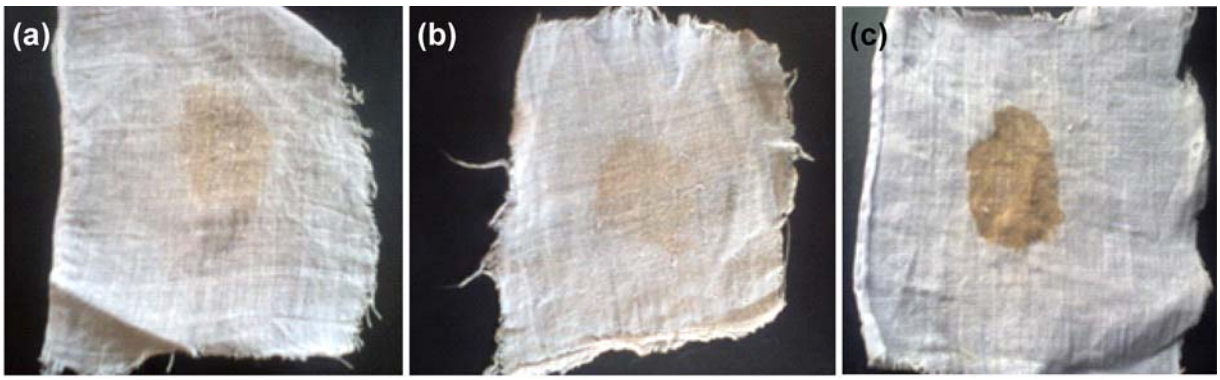

Fig. 3. Removal of blood stain by crude keratinases from wild (a; 3 h) and mutant (b; 2 h) strains; control (c; water).

Miranda et al. 2012; Kadyan et al. 2013) important in molecular biology.

Microbial biodegradation and remediation

Quite a number of industrial activities are leading to the discharge of toxic wastes in the form of heavy metals, antibiotics, hydrocarbons and other chemicals to the environment. The aforementioned compounds constitute nuisance to the ecosystem because of their mutagenic and carcinogenic effects on living tissues, and also endorsing a promotion of evolvement of antibiotic resistant pathogens (Lateef 2004; Adewoye & Lateef 2004a,b; Lateef et al. 2006, 2007). Different biological methods have therefore been adopted to minimize the danger posed by these toxic chemicals, which include microbial biodegradation and remediation. Strains of *B. safensis* and *Micrococcus roseus* have been used in association with some plants to phytoremediate a nickel-polluted site (Motesharezadeh & Savaghebi-Firoozabadi 2011). Similarly, a consortium of *Bacillus* spp. dominated by *B. safensis*, *Bacillus licheniformis* and *Bacillus tequilensis* have been used to efficiently degrade free cyanide (Mekuto et al. 2013) suggesting that the organisms could be used to bioremediate cyanide-contaminated sites. Moreover, Mathe et al. (2012) have reported heavy metal tolerant and antibiotic resistant strain of *B. safensis* as a good aromatic hydrocarbons degrader. In a related study, another heavy metal tolerant and halophilic strain of *B. safensis* has been reported for the biogeochemical cycling of arsenic and

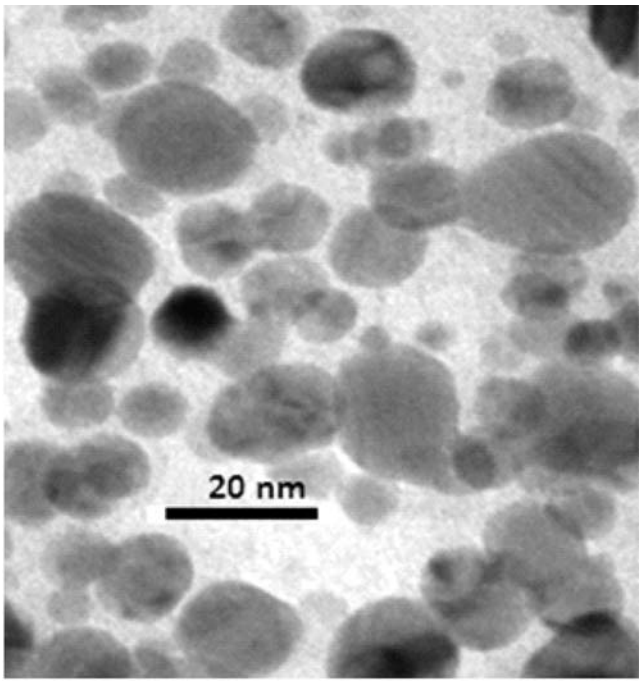

Fig. 4. Transmission electron microscopy micrograph of silver nanoparticles (AgNPs) synthesized using crude keratinase of *B. safensis* LAU 13.

bioremediation of saline sites (Raja & Omine 2012).

Probiotic potential

B. safensis can still be tagged an indispensable machinery in the production of fermented food condiments and as probiotic organism since it has been reported to possess these attributes. Recently, Ahaotu et al. (2013) reported the isolation of *B. safensis* from Ugba, a fermented food product produced from African oil beans. It is a condiment taken as a salad, and as a flavouring agent in soups and sauces. *B. safensis* has also been isolated from Yanyanku and Ikpiru, fermented products from seeds of *Hibiscus sabdariffa* used as additives for the production of fermented food condiments in Benin (Agbobatinkpo et al. 2013), and as well as from Katong, an acid fermented seeds of kapok (*Ceiba pentandra*) used as food condiment in Ghana (Kpikpi et al. 2014). Furthermore, the fermentation of *Houttuynia cordata* by some species of *Bacillus* including *B. safensis* increased its flavonoid compounds with remarkable pharmacological activities (Kwon & Ha 2012). Gerchman et al. (2012) isolated cholesterol degrading strains of *B. safensis* from human cerumen, and the study indicated that the organism could be used as a probiotic especially in the control of body cholesterol. Similarly, Nath et al. (2012a) isolated another strain of *B. safensis* from sweet meat whey that also demonstrated good probiotic potential.

Production of secondary metabolites

B. safensis are also capable of producing secondary metabolites, such as carotenoids (Khaneja et al. 2010), biosurfactant (Porob et al. 2013; Saisa-Ard et al. 2013; Domingos et al. 2015) and arachidonic acid (Goncharova et al. 2013). Carotenoids confer photoprotection in vegetative cells and present very high resistance to ultraviolet radiation in spores (Khaneja et al. 2010). Interest has grown in biosurfactants as good replacement for chemical surfactants in terms of applications, such as biodegradation, detoxification and demonstrable activity under extreme conditions (Rosenberg & Ron 1999). Also, arachidonic acid has been described as a secondary metabolite that plays vital physiological roles (Goncharova et al. 2013), and as an essential component in the formulation of diet, drug, and in the control of heart diseases (Mitmesser & Jensen 2007). In addition, a study has reported a strain of *B. safensis* HA-MS-105 isolated from the sponge *Amphimedon ochracea*, collected from the Red Sea coast of Egypt to have displayed potential cytotoxicity against HepG2 (hepatocellular carcinoma), HCT (colon carcinoma) and MCF-7 (breast carcinoma) cancer cell lines (Aboul-Ela et al. 2012). Thus, *B. safensis* can be termed a good producer of some distinctive category of bioactive secondary metabolites with a wide area of biotechnological applications.

Safety aspect

From the available literature, it appears *B. safensis* is an organism that is safe to handle, as there are no reports linking the bacterium to any primary infection in man, animals and in plants. Its evaluation as a potential probiotic is a further testimony to its safe value. However, the report of occurrence of antibiotic-resistant strains of *B. safensis* calls for caution in handling of the organism, and its application. It is envisaged that with the increasing interest in the biotechnological applications of the bacterium, studies on its proper safety status including cytotoxicity will appear in the future.

Conclusions

Although *B. safensis* was primarily identified as a recalcitrant contaminant in a SAF, its potential in the production of novel biological products continues in progress. It can therefore be concluded that a new vista has been opened in the biotechnological application of strains of *B. safensis* as plant growth-promoting rhizobacteria, bio-control agents, bioremediators, probiotics, and as novel sources of bioactive secondary metabolites and flavonoids. Its metabolites can also be used for the industrial production of prebiotics, and in the green synthesis of nanoparticles. Concerted efforts will be needed to isolate and identify *B. safensis* through the use of high-throughput methodologies, such as MALDI-TOF-MS, and Fourier transform infrared spectroscopy with attenuated total reflectance, which can rapidly and accurately allow the identification of this species. The strains of *B. safensis* can further be improved through rDNA technology and optimization of bioprocesses to extend its frontier as an industrially important bacterium. In this connection, studies on its safety status should be vigorously pursued.

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