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Research Article

The potential of Hungarian bauxite residue isolates for biotechnological applications

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ABSTRACT

Bauxite residue (red mud) is considered an extremely alkaline and salty environment for the biota. We present the first attempt to isolate, identify and characterise microbes from Hungarian bauxite residues. Four identified bacterial strains belonged to the Bacilli class, one each to the Actinomycetia, Gammaproteobacteria, and Betaproteobacteria classes, and two to the Alphaproteobacteria class. All three identified fungi strains belonged to the Ascomycota division. Most strains tolerated pH 8–10 and salt content at 5–7% NaCl concentration. *Alkalihalobacillus pseudofirmus* BRHUB7 and *Robertmurraya beringensis* BRHUB9 can be considered halophilic and alkalitolerant. *Priestia aryabhattai* BRHUB2, *Penicillium chrysogenum* BRHUF1 and *Aspergillus* sp. BRHUF2 are halo- and alkalitolerant strains. Most strains produced siderophores and extracellular polymeric substances, could mobilise phosphorous, and were cellulose degraders. These strains and their enzymes are possible candidates for biotechnological applications in processes requiring extreme conditions, e.g. bioleaching of critical raw materials and rehabilitation of alkaline waste deposits.

1. Introduction

Bauxite residue (BR) (also called red mud) is the alkaline waste of alumina production from bauxite by the Bayer process (hot digestion with caustic soda). It has strongly alkaline characteristics, high salinity, and small particle size and contains both valuable and potentially toxic elements and radioactive components. An estimated 4.4 billion tonnes of BR have been deposited worldwide by 2018 [1], and 200 million tonnes are produced yearly [2]. In 2010, the wall of the cassette X in the BR disposal area in Ajka, Hungary, broke and released caustic bauxite residue suspension, which caused the death of ten people, destroyed houses and flooded approximately 800 hectares of agricultural land [3,4]. Similar mining and metallurgy-related catastrophes, such as the iron mine dam failure releasing 50–60 million m^3 of mud in Brazil in 2015 [5], drew attention to the need to develop appropriate BR deposition, rehabilitation and reuse technologies, e.g., in construction, chemical, environmental, agronomic and metallurgical applications [6–8].

Although BRs are considered extreme environments (e.g., high pH, salinity, toxic metal content), they contain their microflora, mainly extremophilic microorganisms. Extremophiles can live in some of the most aggressive environments on the Earth, e.g. with salinity (halophiles: 0.5–5.2 M NaCl), pH (acidophiles: pH $<$ 4, alkaliphiles: pH $>$ 9) and temperature (psychrophiles: –20 °C to 20 °C, thermophiles: 60–115 °C) [9–11]. Krishna et al. investigated the bacterial diversity of bauxite residue (pH = 11.1) for the first time and identified ten bacterial species in Indian bauxite residue [12]. These strains were alkalitolerant, positive for one or more of the enzyme activities tested, and able to produce organic acids and oxidise a wide range of carbon substrates, which implies their potential application in either bioleaching processes or in the treatment of the BR itself, intending to mitigate its alkalinity [7, 13].

Agnew et al. reported the first strain isolated from Canadian BR and

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identified it as *Evansella vedderi* (formerly *Bacillus vedderi*). This strain was alkaliphilic with a pH optimum of 10 and tolerated NaCl up to 7.5 w/v% [14]. Other strains and their characterisation were reported from Brazil, India and China [15–21]. Most bacterial strains belonged to the genus *Bacillus*, although several have been re-categorised to another genus [22]. Fewer reports are available for fungal strains: two *Penicillium* [17,23] and one *Trichoderma* [16] species have been identified from BRs. Overall bacterial community analysis of deposited BRs in Germany, Ireland and Australia [24–26], India [27], China [28–30], and recently in France was also carried out [31]. Dong et al. also investigated BR's fungal diversity and found Ascomycota as the most dominant phylum [30].

Alkaliphiles are microorganisms that grow optimally above pH 9, often between pH 10 and 12. They can be either obligate alkaliphiles (cannot grow well below or at pH 8) or facultative alkaliphiles (can grow well below or at pH 8). Alkalitolerant microorganisms can grow at alkaline pH, but their optimal growth occurs at pH levels below pH 8 [11,32,33]. Several naturally occurring alkaliphiles are haloalkaliphiles found initially in highly saline soda lakes. They can produce various alkalitolerant enzymes, e.g. proteases, starch-degrading enzymes, cellulases, lipases, xylanases and pectinases and may produce, e.g. siderophores, exopolysaccharides, biosurfactants, organic acids and antibiotics [11,34,35]. Alkaliphiles can maintain cytoplasmic pH much lower than external pH values, which makes them unique in potential biotechnological applications [36]. In environmental biotechnologies, alkaliphiles have been applied to treat, e.g. azo dyes, nitroaromatic compounds, cyanides, petroleum hydrocarbons, metal pollution and alkaline pH [35]. Recent reviews focused on the potential reuse of alkalophilic microorganisms isolated from BR [37–39], showing increasing interest in this topic. These microbes and their enzymes are potential applicants in various biotechnological processes. In some studies, researchers applied for bioleaching microbes isolated from the BR itself [23,40,41]. Bioleaching can be a green alternative to current hydrometallurgical methods to extract critical raw materials, such as scandium (Sc) and rare earth elements (REEs), from BRs, promoting the reuse of this currently deposited waste [42]. They can be used for BR neutralisation [43,44] and as plant growth-promoting bacteria [20]. As indicated by recent research studies, in the foreseeable future, these extremophilic microorganisms could be utilised for biomining rare earth elements in space, specifically on celestial bodies such as Mars or asteroids [45–47].

This paper represents the first attempt in the existing body of literature to systematically explore the microbial composition of a Hungarian BR. Our primary objectives encompassed isolating distinct bacterial and fungal species, focusing on identifying strains endowed with biotechnological utility under extreme environmental conditions. Our investigation contained a comprehensive assessment of these strains, specifically evaluating their resilience to pH, salinity, and temperature variations and their capacity for siderophore and extracellular polymeric substance (EPS) production, nitrogen fixation, phosphorus mobilisation, and cellulose degradation. Our study involved the comparative phylogenetic analysis of our newly isolated strains from Hungarian BR with strains reported previously. Our aim was to find BR-derived microbial isolates that are valuable for the biotechnological industries.

2. Materials and methods

2.1. Sample collection and bauxite residue characterisation

The bauxite residue (BR) samples originated from a former alumina factory in Western Hungary with a humid continental climate, an average temperature of 10–11.5 °C and average rain of 500–800 mm [48]. We investigated three BRs: "A" from the alumina factory – pressure filtered and gypsum treated; "B" from the BR deposit, non-treated, collected in winter; "C" from the BR deposit, non-treated, collected in autumn, stored in the laboratory at room temperature for two years (a BR sample from a previous sampling two years earlier). All BRs were stored at room temperature in plastic bags before sampling for the isolation of microorganisms. We took three homogenised samples from each BR with a sterilised spoon and placed them in a sterile Petri dish. Particular attention was paid to ensuring the samples were taken from the middle of the stored BRs to avoid contamination from the laboratory air. We measured the samples' water content, pH, EC (Hungarian Standard 21,470/2-81:1982), and total metal content. For the water content measurement, 2 g of the sample was added to a jar kept for 24 h at 105 °C. The water content was calculated from the ratio of the original and dried mass of the BR samples. The BRs were air-dried, ground, and sieved (< 2 mm) for the pH, EC, and metal content measurements. The pH and EC were measured by adding 10 mL distilled water to 4 g BR and shaking for 30 min at 200 rpm. pH was measured by Sentix 81 electrode and a WTW (Wissenschaftliche Techniche Werkstätten GmbH) pH 330 instrument, and the EC by Consort C535. For the total metal content measurement, samples were placed in special plastic cups and directly measured by a portable NITON XL3t 600 XRF. For each measurement, we applied three replicate samples.

2.2. Isolation, identification and characterisation of microbial strains

2.2.1. Isolation of microorganisms from BR and strain collection

We used three types of microbial media for strain isolation: alkalophilic Horikoshi agar (10 g/L glucose, 1 g/L yeast extract, 0.2 g/L K_2HPO_4, 0.2 g/L $Mg_2SO_4{\cdot}7H_2O$, 10 g/L Na_2CO_3, 20 g/L agar, pH 9 and 10) for the isolation of alkaliphilic microorganisms [11], nutrient agar (15 g/L peptone, 3 g/L yeast extract, 6 g/L NaCl, 1 g/L dextrose, 15 g/L agar, pH 7.2 and 9) as a general purpose medium and malt agar (20 g/L malt extract, 1 g/L peptone, 1 g/L glucose, 20 g/L agar, pH 5.8 and 9) targeting the isolation of fungi. We used a series of media with the original pH and another series set to alkaline pH (pH = 9), targeting alkalophilic microorganisms. All microbial media used during the series of experiments were sterilised at 121 °C for 25 min in an autoclave. First, 10 mL sterile NaCl solution was added (0.85%) to 1 g BR and was shaken at 180 rpm for 30 min. Afterwards, a ten-fold dilution series was prepared from the BR suspensions and the agar plates were inoculated by the 10^3 and 10^4 dilutions of the suspensions. After incubating the plates for 48 h at 30 °C, we picked morphologically different strains and carried out a series of streaking. We selectively picked strains that exhibited distinct morphological characteristics and proceeded to perform a series of streaking procedures. The resulting strain collection was then preserved in slant media tubes for long-term storage.

2.2.2. Identification of the microbial isolates

We performed DNA extraction to identify the BR isolates. As a physical extraction, one loop of a microbe was added to an Eppendorf tube containing 100 μL dH_2O and 8 μL 1 M TRIS (tris(hydroxymethyl) aminomethane) buffer (pH = 8) and placed in a cell mill for 1 min at 50 Hz. After centrifuging, the cells were denaturated at 98 °C for 5 min, then vortexed and centrifuged for 5 min at 10,000 rpm. We took 70 μL of the supernatant as the extracted and denaturated DNA. If the physical extraction was unsuccessful, as chemical extraction, one loop of a microbe was added to 25 μL 0.5 M NaOH and incubated for 15 min at room temperature. Afterwards, 300 μL dH_2O and 25 μL 1 M TRIS buffer (pH = 8) were added to the suspension and centrifuged for 3 min at 10,000 rpm. We removed 200 μL supernatant containing the isolated DNA [49]. We checked the success of the isolation by agarose gel electrophoresis. The samples were then stored at −20 °C until amplification.

The nearly complete 16S rRNA gene of bacterial strains was amplified with universal 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) [50] primers, while the internal transcribed spacer (ITS) regions of fungal ribosomal DNA were amplified with ITS1-F (5′ CTT GGT CAT TTA GAG GAA GTA A 3′) [51] and ITS4 (5′ TCC TCC GCT TAT TGA TAT GC 3′) [52] primers. The reaction

mixture consisted of 5 μL dNTP mix, 2 μL $MgCl_2$, 2.5 μL buffer, 1 μL Taq DNA polymerase, 0.25 μL 27F primer, 0.25 μL 149R primer, 0.5 μL BSA, 12.5 μL dH_2O, 1 μL template DNA in 25 μL final volume for bacterial strains and 5 μL dNTP mix, 3 μL $MgCl_2$, 5 μL buffer, 1 μL Taq DNA polymerase, 1 μL ITS1-F primer, 1 μL ITS4 primer, 28 μL dH_2O, 5 μL template DNA in 50 μL final volume for fungal strains. The amplification program was as follows: 94 °C for 5 min; 32 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min; and the final extension step at 72 °C for 10 min for bacterial strains and 94 °C for 5 min; 32 cycles of 94 °C for 45 s, 55 °C for 30 s, and 72 °C for 1 min; and the final extension step at 72 °C for 10 min. After Sanger-sequencing (LGC, Berlin, Germany), we analysed the data using the MEGA5 program [53]. We performed the bacterial identifications based on the 16S rRNA sequence similarity using the EzBioCloud 16S-based ID service [54]. The closest relatives (with at least 98% sequence similarity) were identified with a blast search [55] (in the National Center for Biotechnology Information (NCBI) GenBank database. For fungal identifications based on rDNA ITS sequence similarity, we conducted a blast search in the UNITE reference dataset [56]. The closest relatives were identified similarly to the process used for Bacteria. Phylogenetic trees were constructed for Bacteria and Fungi as well. Multiple sequence alignment was carried out using the ClustalW algorithm (with the default settings available in MEGA11 [53], Gap Opening Penalty: 15. Gap Extension Penalty: 6.66). We constructed the phylogenetic trees using the neighbour-joining method with default settings and 1000 bootstrap replications in MEGA11. *Escherichia coli* and *Saccharomyces cerevisiae* sequences were used as outgroups, respectively. Nucleotide sequences of the 16S rRNA or ITS regions were deposited in NCBI GenBank under the accession numbers shown in Table 2 and Supplementary Table S1.

2.2.3. Characterisation of the isolated strains

2.2.3.1. Macro-, micromorphological and biochemical characterisation of the strains. We determined the strains' macro- (e.g., colony colour, surface, edge) and micromorphological (e.g., size, shape, arrangement, endospore formation, mobility) characteristics. Gram-straining was also performed. Subsequently, we conducted a battery of biochemical assays on the strains, including tests for oxidase activity, aerobic and anaerobic glucose degradation, aerobic arabinose and xylose degradation, citrate and urease utilisation, nitrate reduction, as well as assessments of starch and gelatine degradation. These methodologies were adopted from established protocols [57–59].

2.2.3.2. pH, salt and temperature tolerance of the strains. We carried out the tolerance studies of the BR-derived strains by modifying the methods described by Kutasi et al. [60]. We measured the strains' pH (5–10) tolerance in a modified nutrient media (10 g/L peptone, 3 g/L yeast extract, 6 g/L NaCl, 5 g/L glucose). To test the pH tolerance, we applied various buffer solutions [61]: citrate buffer for pH 5 and 6, phosphate buffer for pH 7, Tris–HCl buffer for pH 8 and carbonate-bicarbonate buffer for pH 9 and 10. We measured the salt (0–15%) tolerance in modified nutrient media (10 g/L g peptone, 3 g/L yeast extract, 5 g/L glucose) for bacteria and malt media (20 g/L malt extract, 1 g/L peptone, 5 g/L glucose) for fungi. The pH of the media has been set between 7 and 8. The following salt concentrations were tested: 0%, 0.6%, 1%, 3%, 5% and 7% NaCl. We measured temperature tolerance (5–40 °C) in the previously explained media at 5 °C (in a fridge), 20 °C (room temperature), 30 °C and 40 °C in thermostats.

For the tolerance studies, we added one loop of 24-hours-old microbes (cultured at 30 °C on slant agar) to 4.5 mL sterile tap water. The tests were conducted in three replicates and an uninoculated control for each pH. After homogenisation, we added 100 μL of the microbial suspension to a 5 mL test solution in a tube. Afterwards, we measured the optical density (OD) at 24 and 48 h (tubes incubated at 30 °C for pH and salt-tolerance) at 490 nm by a DIALAB EL-800 ELISA (Dialab GmbH, Austria) plate reader by adding 150 μL of the inoculated test solution to each well of a transparent microplate. The measured sample OD was corrected by the control OD. Heatmap diagrams of pH, NaCl concentration and temperature tolerance were performed with the seaborn (0.11.2) and matplotlib (3.5.2) Python packages.

2.2.3.3. Siderophore production of the strains. Siderophores are small molecular weight iron-chelating organic molecules that can also chelate other ions [62]. We examined the siderophore production capacity of the strains according to Schwyn and Neilands, as described by Louden et al. [63] (we used tryptone instead of casamino acid). We divided the agar plates into three parts and inoculated each part (three replicates) with one loop of 24 h or 48 h old microbe (cultured at 30 °C on slant agar). The plates were incubated for 48 h (30 °C) and the siderophore production was detected by the yellow or orange colour changes on the blue agar plates. The siderophore production capacity of the strains was evaluated qualitatively based on the amount of colour development in the Petri dishes. They were categorised as low, moderate, and intensive siderophore-producing strains.

2.2.3.4. EPS production of the strains. Extracellular polymeric substances (EPS) are polymers that mainly protect microbial cells [64]. We assessed the EPS production capacity of the strains according to Kutasi et al. [60]. We inoculated 30 mL media (0.51% KNO_3, 0.1% K_2HPO_4, 0.08% KH_2PO_4, 0.04% $MgSO_4{\cdot}7H_2O$, 0.002% $FeSO_4{\cdot}7H_2O$, 0.001% $CaCl_2$, 0.001% $MnSO_4$, 0.1% NaCl, 0.01% yeast extract, 0.01% tryptone, 0.01% bacto peptone, l mL/L salt solution containing 0.3 g H_3BO_3, 0.2 g $CoCl_2{\cdot}6H_2O$, 0.1 g $ZnSO_4{\cdot}7H_2O$, 30 mg $Na_2MoO_4{\cdot}2H_2O$, 20 mg $NiCl_2{\cdot}6H_2O$, 10 mg $CuSO_4{\cdot}5H_2O$, in 1 L 1 N HCl, pH = 7.0) with three loops of 24 h or 48 h old microbe (cultured at 30 °C on slant agar) in 50 mL Falcon tubes. The Falcon tubes were shaken at 300 rpm at 30 °C for 7 days and then centrifuged at 5000 rpm at 20 °C for 45 min. We added 15 mL supernatant to 15 sterile distilled water and incubated the flasks at 40 °C with magnetic stirring for 2 h. After incubation, we added 3 times the amount of 2-propanol. The EPS production was detected by opaque colour, bubbling and/or the change in viscosity. We evaluated the EPS production quantitatively based on opacity and the amount of bubble development. They were categorised as low, moderate, and intensive EPS-producing strains.

2.2.3.5. Nitrogen-fixing, phosphorous mobilising and cellulose-degrading potential of the strains. Nitrogen-fixing, phosphorous mobilising and cellulose-degrading potential were investigated on Ashby (20 g/L mannitol, 5 g/L $CaCO_3$, 0.2 g/L KH_2PO_4, 0.2 g/L $MgSO_4$ 7 H_2O, 0.1 g/L K_2SO_4, 16 g/L agar, pH = 7.4), Pikovskaya (10 g/L glucose, 5 g/L Ca $(H_2PO_4)_2$, 0.5 g/L yeast extract, 0.5 g/L $(NH_4)_2SO_4$, 0.2 g/L KCl, 0.1 g/L $MgSO_4{\cdot}7$ H_2O, 0.0001 g/L $MnSO_4$, 0.0001 g/L $FeSO_4$, 16 g/L agar, pH = 7.4), and Fuller-Norman (10 g/L yeast extract, 10 g/L cellulose, 1/L g $NaNO_3$, 1 g/L K_2HPO_4, 0.5 g/L KCl, 16 g/L agar, pH = 7.0) agar plates. The composition of the nutrient media Ashby, Pikovskaya, and Fuller-Norman was determined based on Zhang et al., Nautiyal and Vasiliauskiene et al. respectively [65–67]. We inoculated the agar plates with 100 μL microbial suspension (1 loop in 4.5 mL sterile water) of 24–48 h old microbial cultures at 10^3, 10^4 and 10^5 dilutions. We measured the number of colonies developed after 3 and 7 days in the case of the Ashby and the Pikovskaya agars and after 3, 5, 7 and 10 days on the Fuller-Norman plates. A strain was considered + for the investigated characteristics if we saw a concentration-dependent number of colonies forming on the plates.

2.3. Statistical analysis

We performed an analysis of variance (ANOVA) using StatSoft® Statistica 7. The level of significance was established at $p < 0.05$. Fisher's Least Significant Difference (LSD) test was used for post-hoc

comparison. Values followed by the same letter indicate no significant differences in the calculated values at the level of $p < 0.05$.

3. Results

3.1. Bauxite residue characteristics

The gypsum-treated BR (BR A) had the lowest pH, the highest electrical conductivity and the lowest moisture content (Table 1). There were some statistically significant differences in the metal and metalloid content of the BRs. BR A had the lowest Cu, Mo, Mn and V content, while BR C had the lowest As, Ca, Fe, Ni and Pb content. The Hungarian BRs have a total of 40,310–48,310 mg/kg Al and 13,723–14,634 mg/kg Na content (measured after Aqua Regia digestion by ICP-OES, [68])

3.2. The identified microbial BR isolates

We sequenced the 16S rRNA gene of twelve bacterial isolates, and based on these partial sequences, we identified twelve bacterial strains at the species level (Table 2, Supplementary Table S1) with 98–100% identity to the top-hit strains in the EzBioCloud 16S rRNA identification database. We performed a phylogenetic analysis with the close relatives, and the strains formed five distinct phylogenetic groups on the tree (Fig. 1). Six strains belonged to the Bacilli class, one each to the Actinomycetia, Gammaproteobacteria, and Betaproteobacteria classes, and three to the Alphaproteobacteria class. Based on the high similarity and close relation on the phylogenetic tree, we have chosen a representative strain from each highly similar group as we hypothesised these were identical strains isolated on different media (see Table S2) from different sources (BRHUB9 (from BR C isolated on nutrient agar), BRHUB10 (BR A, malt agar) and BRHUB11 (BR B, malt agar); BRHUB1 (BR A, nutrient) and BRHUB12 (BR B, alkaline nutrient)). We chose to present the BRHUB9 and BRHUB1 strain's characteristics. Data on BRHUB10, BRHUB11 and BRHUB12 were added to Table S1.

We sequenced the internal spacer region of the nuclear rRNA of five fungal isolates, and based on these sequences, we identified five fungal strains at the genus level with 100% identity (Table 2, Table S1), all belonging to the Ascomycota division. We created a phylogenetic tree similar to the bacteria with the closest relatives, and the identified strains formed two distinct phylogenetic groups on the tree (Fig. 2). Four belonged to the Eurotiomycetes class, and one to the Sordariomycetes class. Based on the high similarity and the close relationship, we chose BRHUF2 as a representative strain and added data on BRHUF4 and BRHUF5 to Table S1. These three strains were isolated from the same BR C on malt (BRHUF2 and BRHUF4) and nutrient (BRHUF5) media.

Table 1
Chemical characteristics of the BRs. Values followed by the same letter indicate no significant differences in the measured parameter between the BRs ($p < 0.05$).

Parameter	BR A	BR B	BR C
pH	8.8 ± 0.1 a	8.9 ± 0.0 b	9.1 ± 0.1 c
EC (μS/cm)	119 ± 11 a	926 ± 9 b	668 ± 7 c
Moisture (%)	19.7 ± 0.2 a	32.8 ± 0.1 b	26.0 ± 0.3 c
Total metals and metalloids (mg/kg)			
As	130 ± 6 a	119 ± 1 b	98.2 ± 1.1 c
Ca	69,129 ± 5689 a	64,127 ± 2741 a	27,133 ± 6118 b
Cr	365 ± 25 a	343 ± 12 a	357 ± 8 a
Cu	100 ± 6 a	134 ± 14 b	132 ± 2 b
Fe	342,656 ± 21,944 a	403,193 ± 3539 b	276,504 ± 12,614 c
Mo	5.19 ± 2.32 a	12.4 ± 14.7 b	10.6 ± 1.3 b
Mn	2174 ± 143 a	5857 ± 262 b	2903 ± 227 c
Ni	366 ± 47 a	400 ± 33 a	157 ± 12 b
Pb	115 ± 0 a	150 ± 5 b	104 ± 1 c
Ti	16,570 ± 1239 a	22,812 ± 663 b	14,525 ± 1562 a
V	406 ± 21 a	595 ± 27 b	539 ± 10 c

3.3. Characteristics of the isolated strains

Strains were isolated and identified from all three BRs and on all kinds of media with various pHs. We present the micro and macromorphological characteristics of the strains and some biochemical features in the supplementary (Tables S2 and S3, Figure S1).

3.3.1. pH, salt and temperature tolerance of the strains

We characterised the microbial strains based on their pH, salt and temperature tolerance (Fig. 3). Most strains had a broad pH tolerance from pH 5–10 (the range measured). The BRHUB1 and BRHUB7 strains did not tolerate a pH below 7. BRHUB5, BRHUB8 and BRHUF2 showed high reproduction at pH 8 or 9. BRHUB1, BRHUB6, BRHUB8, BRHUF1 and BRHUF3 showed intensive replication at NaCl content of up to 3% (0.51 M) and tolerated salt content of up to 5% (0.86 M). Strain BRHUB7 reproduced only at higher salt concentrations (3–5%). Strains BRHUB2, BRHUB5, BRHUB6, BRHUB7, BRHUB9, BRHUF1, BRHUF2 and BRHUF3 tolerated salt content of up to 7% (1.20 M). Three bacterial strains (BRHUB2 up to 12%, 2.05 M, BRHUB7 up to 10%, 1.71 M and BRHUB9 up to 15%, 2.57 M) and all fungal strains (up to 15%) could also tolerate higher salt content (data not shown in the Figure). The optimal temperature for growth was between 20 and 40 °C for most strains. Strains BRHUB6, BRHUB8, BRHUF1, BRHUF2 and BRHUF3 could grow at 5 °C. BRHUB1, BRHUB2, BRHUB4, BRHUB6, BRHUB7, BRHUB8 and BRHUF3 could grow at 50 °C.

3.3.2. Siderophore and EPS production, nitrogen-fixing, phosphorous mobilising and cellulose-degrading potential of the strains

Most strains produced siderophores and EPS (Table 3, Figure S2 and S3). The best bacterial siderophore-producing strain was BRHUB6, followed by BRHUB1 and BRHUB3. BRHUB2 and BRHUB5 made EPS in the highest amount. All fungal strains produced a high amount of siderophores and EPS. We found three nitrogen-fixing strains (BRHUB2, BRHUB4 and BRHUB6), but several strains could mobilise P and degrade cellulose.

4. Discussion

4.1. Bauxite residue characteristics

The Hungarian BRs have an alkaline (pH 8.5–9.0) or strongly alkaline (pH > 9.0) pH and can be considered non-saline (EC < 2 mS/cm) [69]. The gypsum-treated and pressure-filtered BR had the lowest pH, moisture content, and EC. Gypsum can be applied for safer deposition and BR rehabilitation [70]. Considering the toxic metal content of the BRs, As (HQC: 75 mg/kg) and Ni (HQC: 200 mg/kg) were above the Hungarian Quality Criteria (HQC) for sewage sludge (HG 50/2001). Based on these characteristics, Hungarian BRs may be a less harsh environment as habitat for microorganisms compared to other BRs worldwide (e.g. Indian BR with pH 11.1, EC 4.9 mS/cm [12], Chinese BR with pH 10.1–12.9, EC 9.3–21.8 mS/cm [71], Brazilian BR pH 10.53–10.73 [15]) but similar to other European BRs (e.g. French BR pH 8.7–11.9, EC 0.29–2.53 mS/cm [31]). These differences may originate from the bauxite processed, the process parameters, post-treatments, environmental parameters, storage time and conditions [8,72]. According to Santini et al. when investigating various BRs from Germany, Ireland, and Australia, salinity and total alkalinity mostly influence the microbial community structure in BRs [7].

4.2. Characteristics of the strains isolated from Hungarian BRs

We isolated, identified and characterised nine bacterial and three fungal strains. We found strains in all three BR types and isolated them on various microbial media with acidic/neutral/alkaline pH. This showed that cultivable strains could be found in gypsum-treated, not deposited (BR A), non-treated, deposited at an open storage facility (BR

Table 2
Top-hit taxons of the identified bacterial and fungal strains. Similarity values represent the identity with the top-hit taxon's 16S rRNA or nuclear ribosomal ITS region, respectively.

Name	GenBank ID	Top-hit taxon	Top-hit strain	GenBank ID of top-hit strain	Similarity (%)	Top-hit taxonomy	Completeness (%)
BRHUB1	OR506201	*Brucella pseudogrignonensis*	CCUG 30,717	NNRM01000012.1	98.89	Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Brucellaceae; Brucella	89.50
BRHUB2	OR506211	*Priestia aryabhattai*	B8W22	EF114313.2	100.00	Bacteria; Firmicutes;Bacilli; Bacillales; Bacillaceae; Priestia	98.60
BRHUB3	OR506249	*Halalkalibacter krulwichiae*	AM31D	CP020814.1	98.71	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Halalkalibacter	100.00
BRHUB4	OR506255	*Massilia timonae*	CCUG 45,783	AGZI01000009.1	98.38	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Oxalobacteraceae; Massilia	97.90
BRHUB5	OR506259	*Nesterenkonia massiliensis*	NP1	HG005180.1	99.86	Bacteria; Actinobacteria; Actinomycetia; Micrococcales; Micrococcaceae; Nesterenkonia	95.40
BRHUB6	OR506262	*Stutzerimonas stutzeri*	ATCC 17,588	CP002881.1	99.72	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Stutzerimonas	98.00
BRHUB7	OR506272	*Alkalihalophilus pseudofirmus*	DSM 8715	X76439.1	99.93	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Alkalihalobacillus	98.70
BRHUB8	OR506275	*Brevundimonas naejangsanensis*	DSM 23,858	FJ889576.2	99.63	Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas	77.80
BRHUB9	OR506276	*Robertmurraya beringensis*	BR035	ATXN01000003.1	99.69	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Robertmurraya	64.80
BRHUF1	OR506340	*Penicillium chrysogenum*	E20401_ITS	MK267450	100	Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiomycetidae; Eurotiales; Aspergillaceae; Penicillium; Penicillium chrysogenum species complex	97.54
BRHUF2	OR506355	*Aspergillus* sp.	xy02	MG798655.1	100	Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Eurotiomycetes; Eurotiomycetidae; Eurotiales; Aspergillaceae; Aspergillus	95.71
BRHUF3	OR506382	*Acrostalagmus luteoalbus*	JAC12603	MK432726.1	100	Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreomycetidae; Glomerellales; Plectosphaerellaceae; Acrostalagmus	92.08

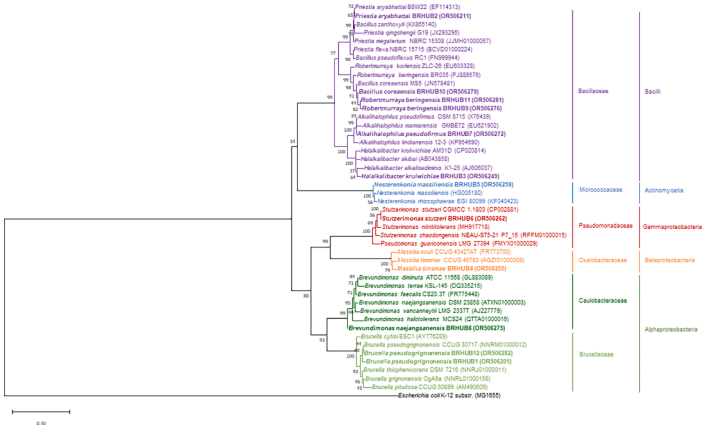

Fig. 1. Phylogenetic tree based on the 16S rRNA sequences of the isolated bacterial strains and some of their closest relatives. The tree was constructed using the neighbour-joining method with 1000 bootstrap replicates, and *E. coli* was used as the outgroup to root the tree. The bar represents 1 substitution per 10 nucleotides. Numerical values indicate the percentile of the bootstrap replicates. NCBI GenBank IDs are shown after the strain names in brackets. Colours represent the respective taxonomies.

B), and non-treated, deposited, laboratory stored (BR C) bauxite residues. Most identified strains originated from the relatively freshly sampled, long-term deposited BR (BR B). All identified genera had a representative strain in BR B. In most studies, the isolated strains also originated from open deposits of BRs [12,17,23,44] or BR ponds [13,15,73].

We isolated four species belonging to Bacilli. Other authors also found several Bacilli strains in BRs from all over the world, e.g. USA [13], India [12], Brazil [15], and China [18,19,21,44,74]. Santini et al. and Wu et al. found Firmicutes to be one of the most dominant phyla in BRs [25,28]. Yadav et al. have shown that *Bacillus* and *Bacillus*-derived genera are tolerant to low and high pH and temperature, salt and low moisture are present in niche-specific and extreme environments [75].

Priestia aryabhattai (BRHUB2) (synonym: *Bacillus aryabhattai*) is a salt-tolerant (up to 12 w/v%) mesophilic strain found in various environments [76]. Our strain also tolerated salt up to 12%, had a wide pH optimum, and produced a high amount of EPS. Some other *Priestia aryabhattai* strains (endophytic from leaf and stem parts of *Triticum aestivum* L.) have been proven to be applicable as plant growth-promoting rhizobacteria (PGPR) inducing salt tolerance in plants [77]. *Robertmurraya beringensis* (BRHUB9) (synonym: *Bacillus beringensis*) is a psychrotolerant strain originally isolated from the Bering Sea [78,79]. Bibi et al. found *R. beringensis* associated with halophites [80] and Jacob et al. in hot springs [81], showing that they are halophilic and have a wide range of temperature tolerance. Our strain tolerated pH up to 10 and grew at a salt content of 12–15%, a good

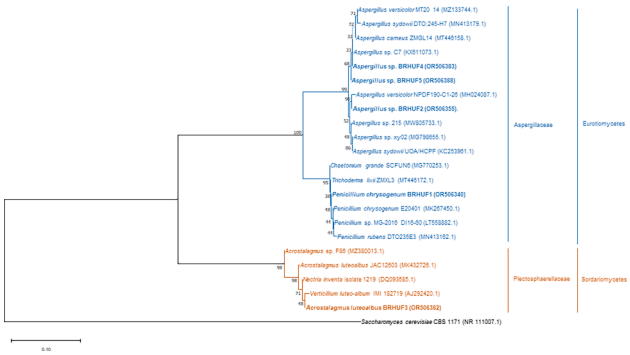

Fig. 2. Phylogenetic tree based on the nuclear rRNS ITS region of the isolated fungi strains and some of their closest relatives. The tree was constructed using the neighbour-joining method with 1000 bootstrap replicates, and *S. cerevisiae* was used as the outgroup to root the tree. The bar represents 1 substitution per 10 nucleotides. Numerical values indicate the percentile of the bootstrap replicates. NCBI GenBank IDs are shown after the strain names in brackets. Colours represent the respective taxonomies.

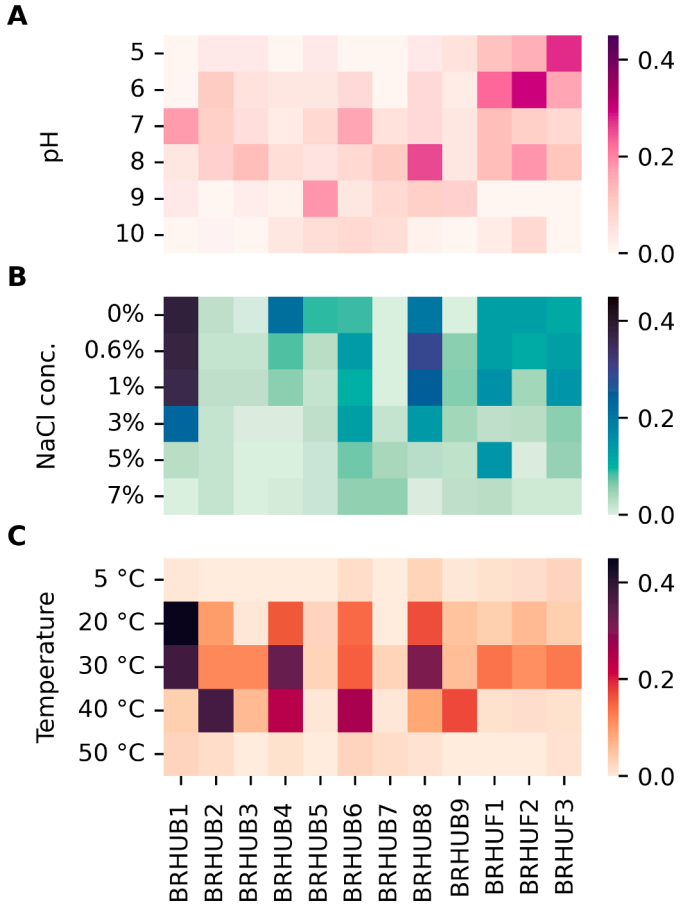

Fig. 3. Heat map diagrams of pH (A), salt (B) and temperature (C) tolerance of the isolated BR bacterial and fungal strains. The colours represent the OD values measured under the corresponding conditions.

candidate for extremophilic characteristics (alkaliphiles pH > 9 [11], halophiles 0.5–5.2 M NaCl [10]. *R. beringensis* can be applied for biohydrogen production [82], and its close relative, *Bacillus coreaensis*, is capable of xylan hydrolysis [83]. *Alkalihalophilus pseudofirmus* (BRHUB7) (synonym: *Alkalihalobacillus pseudofirmus, Bacillus pseudofirmus*) is alkalophilic and halotolerant, first isolated from soil and then found in various environments [76]. Our strain grew at pH 7–10 and tolerated 5–10% NaCl concentration. It can produce feather-degrading enzymes [84] and alkaline protease [85] or even repair concrete cracks [86]. *Halalkalibacter krulwichiae* (BRHUB3) (synonym: *Alkalihalobacillus krulwichiae, Bacillus krulwichiae*) was isolated from the soil [87]. It is a mesophilic, obligate alkalophilic bacterium [88]. However, our strain tolerated pH from 5 to 9 with optimum pH = 8 and showed low tolerance to salt. It intensively produced siderophores.

Nesterenkonia massiliensis (BRHUB5) is a Gram+ mesophilic bacteria isolated from human faeces [89]. Our strain has a wide range of pH and salt tolerance. Several *Nesterenkonia* sp. strains were isolated from hypersaline or alkaline environments [90], Borsodi et al. isolated a novel haloalkaliphilic *Neseterenkonia pannonica* strain from a soda pan (pH 9.1) located in Kiskunság National Park, Hungary [91]. Haloalkaliphilic *Nesterenkonia* strains have been reported to produce lipases and siderophores [92]. In several BRs, Actinobacteria was among the most dominant species [25,28,31].

Stutzerimonas stutzeri (BRHUB6) (synonym: *Pseudomonas stutzeri*) is a well-known species isolated from various environments, and its presence is almost universal due to its remarkable physiological and biochemical diversity and flexibility. They have been isolated from the marine environment and salt marshes (our strain could reproduce well at up to 5% NaCl and tolerate 7% salt in the media), and they are resistant to metals, such as Al, As, Ni, etc. [93]. Several authors have found Gammaproteobacteria [28,31] and *Pseudomonas* species [12,13, 20,27] in BRs. Therefore, we anticipated their presence in the Hungarian BR. Our strain's pH optimum was pH = 7, but it could tolerate pH = 10. Based on the literature data, this species does not grow under pH 4.5 [93]. Our strain's optimum growth was at 40 °C, but they grow well at 20–40 °C. According to the literature, they are mesophilic [94]. BRHUB6 strain was able to fix nitrogen, mobilise P and degrade cellulose. After years of controversy, it is clear that some *Stutzerimonas stutzeri* strains can fix nitrogen. They are capable of P mobilisation, and due to their genetic adaptability, they can degrade several biogenic and xenobiotic compounds [93].

Massilia timonae (BRHUB4), a mesophilic bacteria, has been isolated from human sources and found in soils and air [95]. It can live associated with root noodles [96] or in the water of a cooling tower [97]. Our strain was also mesophilic and did not tolerate high salt content (optimum: 0% salt in the media), but it could live in a wide pH range (pH = 6–10). It was also able to produce siderophores, fix nitrogen, mobilise P and degrade cellulose. *M. timonae* can produce extracellular endochitinases [98], amylolytic enzymes [99] and chitinase [100].

Brucella pseudogrignonensis (BRHUB1) (synonym: *Ochrobactrum pseudogrignonense*) was first isolated from human blood but found in the environment at various places. They tolerate salt up to 5% and grow at 30 °C [101]. Our strain had a pH optimum at pH = 7 and was mesophilic, with optimal growth between 20 and 30 °C. *B. pseudogrignonensis* has plant growth-promoting traits and can alleviate salt and other stressors in plants [102–104]. It also has potential as a biocontrol agent [74]. Chaturvedi and Verma isolated a *B. pseudogrignonensis* strain from a copper mine wastewater, proving its malachite green degrading potential [105]. *Brevundimonas naejangsanensis* (BRHUB8) strains have been isolated from all environments, originally from Korean soil. Their pH optimum is pH = 8, they tolerate salt concentrations up to 4%, and their optimal temperature is 30 °C [106]. Our results followed the literature data. *B. naejangsanensis* strains can biodegrade various compounds, such as antibiotics [107,108] and pesticides [109] even in alkaline environments [109]. Santini et al. showed that Proteobacteria was the most dominant phyla (40–80% relative abundance) in BRs [25]. Macías-Pérez et al. found that Alphaproteobacteria was present in 12% (relative abundance among the most abundant bacterial taxa identified) in a BR from Provence [31].

Based on the phylogenetic tree representing bacterial strains isolated from other BRs, we can conclude that we found and identified the most diverse microflora in the Hungarian BR compared to other BRs (Fig. 4). Differences may originate from the variations in microbial habitat

Table 3
Biotechnologically interesting characteristics of the bacterial and fungal strains. Siderophore and EPS production categories: low, moderate and intensive production. Strains were either capable (+) or incapable (-) of nitrogen fixation, P mobilisation or cellulose degradation.

Characteristics	BRHUB1	BRHUB2	BRHUB3	BRHUB4	BRHUB5	BRHUB6	BRHUB7	BRHUB8	BRHUB9	BRHUF1	BRHUF2	BRHUF3
Siderophore production	moderate	low	moderate	moderate	low	intensive	n.d.	low	low	moderate	intensive	intensive
Siderophore type (colour)	orange	yellow	yellow	yellow	yellow	orange	n.d.	yellow	yellow	yellow and orange	yellow	yellow
EPS production	low	intensive	n.d.	none	intensive	n.d.	n.d.	moderate	low	moderate	moderate	moderate
Nitrogen fixation	–	+	–	+	–	+	–	–	–	n.a.	n.a.	n.a.
P mobilisation	+	+	+	+	–	+	+	–	–	–	–	–
Cellulose degradation	+	+	+	+	+	+	–	+	+	–	–	–

n.d.: not determined, n.a.: not applicable.

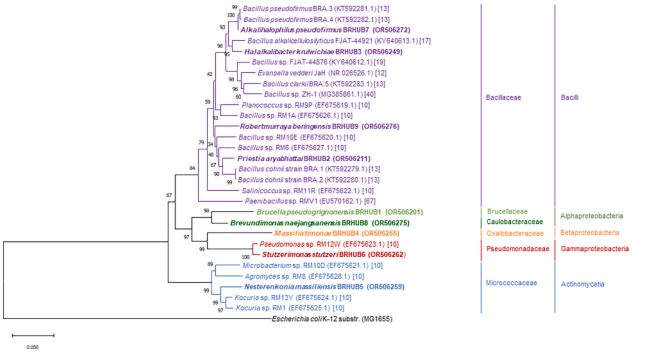

Fig. 4. Phylogenetic tree based on the 16S rRNA sequence of the bacterial strains from Hungarian BRs and strains originating from other BRs. The tree was constructed using the neighbour-joining method with 1000 bootstrap replicates, and *E. coli* was used as the outgroup to root the tree. The bar represents 5 substitutions per 100 nucleotides. Numerical values indicate the percentile of the bootstrap replicates. NCBI GenBank IDs are shown after the strain names in brackets. Colours represent the respective taxonomies.

characteristics (e.g. pH, salt content) between the BRs as explained in Chapter 4.1. Although several Bacilli strains, a few Actinomycetia and one Gammaproteobacteria have been isolated from BRs previously, the isolation and identification of *Brucella* sp., *Brevudiomonas* sp. (Alphaproteobacteria) and *Massilia* sp. (Betaproteobacteria) from BR is a novelty. Caulobacteraceae was found by Schmalenberger et al. in rehabilitated bauxite residue deposition areas but not in the untreated BR [24]. Santini et al., Ke et al. and Macías-Pérez et al. declared Proteobacteria to be one of the most dominant phyla in BRs [25,29,31]. Wu et al. found Alphaproteobacteria (relative abundance of 11–14%) and a small amount of Betaproteobacteria (relative abundance of 3–7%), mainly in old, weathered BR [28]. Macías-Pérez et al. gained similar results on the relative abundance of these two classes in one of the BRs investigated [31]. Based on these metagenomics studies, the members of the Proteobacteria phylum are inhabitants of BRs, but their relative abundance strongly depends on the BR type.

Regarding fungi strains, *Aspergillus* and *Penicillium* species are widely distributed in the environment and applied in the pharmaceutical, agricultural, food, and medical industries to produce enzymes, organic acids, and bioactive compounds [110–113]. *Penicillium chrysogenum* (BRHUF1) is a commonly occurring mold in indoor, terrestrial and marine environments [112,114], which produces penicillin [115] and siderophores [116,117]. Qu et al. isolated a *Penicillium tricolor* strain from Chinese BR and used it to bioleach valuable elements, including rare earth elements from the BR [41,71]. Liao et al. also applied a *Penicillium oxalicum* strain from BR to reduce the alkalinity of the BR. In both cases, the authors used the acid-producing capacity of these fungi in their technology [17].

Our *Aspergillus* strain (BRHUF2) was closely related to *Aspergillus versicolor* and *Aspergillus sydowii*. *A. versicolor* can be found in various environments, including indoor air, soil, marine environment and food, e.g. cheese [110,111]. Similar to our strain, some *A. versicolor* strains tolerate high salt content [118]. *Aspergillus sydowii* also grows in various environments, such as soil, sea and food and is considered a human and zoonic pathogen [119].

Acrostalagmus luteoalbus (BRHUF3) is a less well-known fungi species considered rare in the environment. However, researchers found them in freshwater [120], on outdoor walls [121], in plants [122,123] or in extreme environments, such as the deep sea [124] and the Antarctic [125]. Grum-Grzhimaylo et al. isolated the *Acrostalagmus luteoalbus* strain from soils surrounding soda lakes in Asia and Africa [32]. The authors characterised the strain as strongly alkalitolerant, and its growth was positively affected by the presence of Na^+ ions. We presume that *A. luteoalbus* species are more common in the environment, even in extreme habitats than previously considered.

Although metagenomic analysis of fungi in BRs has been rarely conducted, both Santini et al. and Dong et al. showed that Ascomycota was the most dominant phyla (50–100% and 86%, respectively) [25,30]. Based on Dong et al. study [30], our strains were not among the most prevalent fungi genera. However, fungi strains isolated from other BRs also belonged to these two Aspergillaceae and Plectosphaerellaceae families (Figure S4), meaning that these might be the easiest to isolate and identify.

4.3. Alkaliphile/alkalitolerant and halophile/halotolerant characteristics of the stains

All strains isolated from Hungarian BR (except for BRHUF3) could grow at pH 9 and pH 10, meaning that they can grow in an alkaline environment and can be considered alkaliphilic or alkalitolerant [11,32,126]. Regarding optimal growth, the isolated bacteria would prefer to grow below pH 9, thus, we categorised them as alkalitolerant. Fungal strains BRHUF1 and BRHUF2 preferred pH 5–6 but grew at pH 8 and pH 10, meaning that they are also alkalitolerant. None of the strains were obligate alkaliphiles, as they could grow at neutral pH or below.

Microbes tolerating 0.2–0.5 M NaCl, which is 1.2–2.9% NaCl, can be categorised as weakly halophilic, while moderately halophilic grow at 0.5–2.5 M (3–14.4%), borderline extremely halophilic at 1.5–4 M (8.8–29.2%) and extreme halophilic at 2.5–5.2 M (14.4–30.4%) [10,127]. Halophiles require salt to grow, unlike halotolerant microorganisms, which can grow without salt. Halotolerants tolerate salt content up to 2.5 M (14.4%) or even more [127]. All strains tolerated 5% NaCl (or above) (except for BRHUB3 and BRHUB4); thus, they can be categorised as moderately halophilic, but only BRHUB3, BRHUB7, and BRHU9 required salt for their growth. Therefore, we categorised bacterial strains as moderately halotolerant, except for BRHUB2, BRHUB7, and BRHUB9, which could grow at up to 12, 10 and 15%, respectively. We can call them borderline extremely halophilic (BRHUB7 and BRHUB9). All fungal strains are moderately halotolerant.

Based on their tolerances, we can categorise *Priestia aryabhattai* BRHUB2, *Penicillium chrysogenum* BRHUF1, and *Aspergillus* strain BRHUF2 as halotolerant and alkalitolerant microorganisms. *Alkalihalophilus pseudofirmus* BRHUB7 and *Robertmurraya beringensis* BRHUB9 are halophilic and alkalitolerant bacterial strains.

4.4. Production of biotechnologically interesting compounds by the strains

Siderophores enhance the uptake of iron, which is insoluble and inaccessible in the ferric form at environmental conditions. Siderophore-producing strains may be applied for biotechnological purposes, such as bioremediation of metal-polluted soils, in biosensors, plant growth promotion, biocontrol of pathogens, in the food industry and medical applications [128,129]. *Stutzerimonas stutzeri* (*Pseudomonas stutzeri*) is well-known for producing various types of siderophores, such as pyoverdines, desferriferrioxamines, and catechol-like siderophores. These siderophores ensure essential metals' availability and metal resistance for this species [93]. Gunasekaran et al. found that a close relative of *R. beringensis, Bacillus coreaensis,* was sodic tolerant and could produce siderophores and EPS [130]. Most fungi produce a variety of different siderophores [131]. Our fungal strains were all strong siderophore producers.

Exopolymeric substances (EPS) have a wide range of applicability in the diary, pharmaceutical and textile industry, agriculture and bioremediation. The ability of EPS production is widespread in nature, e.g. *Pseudomonas* sp. and *Bacillus* sp. are EPS producers [132]. Harmiawan et al. showed that *Massilia timonae* had a high propensity to form EPS [133].

Microbes can solubilise various elements from soils, which is essential, e.g., for mobilising nutrients required by plants, such as P or K, or metals in remediation technologies, such as phytoextraction [134]. For example, Xu et al. found a *Massilia timonae* strain to mobilise insoluble Cd from metal-polluted soil [135] and other plant growth-promoting rhizobacteria, e.g. *Bacillus* sp. and *Pseudomonas* sp., have been applied in assisted phytoremediation [134]. A *Priestia aryabhattai* strain isolated from cassava plant rhizosphere showing zinc and phosphate mobilising capacity enhanced the growth of green soybean [136]. Another *P. aryabhattai* strain capable of P mobilisation, siderophore and EPS production alleviated salinity stress in wheat plants [137]. *Brevundimonas naejangsanensis* strain with siderophore-producing and potassium solubilising capacity has been used in a consortium for plant growth promotion [138]. These findings underline the potential usability of BR-derived strains in biotechnologies, including agricultural applications.

5. Conclusions

Our research identified and characterised nine bacterial and three fungal strains isolated from Hungarian bauxite residue (BR). We classified four bacterial strains within the taxonomic classes Bacilli, and the others belonged to Actinomycetia, Gammaproteobacteria, Betaproteobacteria and Alphaproteobacteria, while all fungal isolates belonged to Eurotiomycetes and Sordariomycetes classes. Compared to previous studies, we could isolate, identify and characterise the most diverse group of bacteria and fungi from BR. Novel isolated strains belonged to the Alphaproteobacteria and Betaproteobacteria classes. Similarly to previous results, Bacilli and Aspergillaceae strains had the broadest pH and salt tolerance range. *Alkalihalophilus pseudofirmus* BRHUB7 and *Robertmurraya beringensis* BRHUB9 are halophilic and alkalitolerant strains, while *Priestia aryabhattai* BRHUB2, *Penicillium chrysogenum* BRHUF1 and *Aspergillus* strain BRHUF2 as halotolerant and alkalitolerant microorganisms. Most of the isolates exhibited the production of siderophores and extracellular polymeric substances, demonstrated the ability to mobilise phosphorus, or were capable of cellulose degradation. Our study highlights the importance of isolating extremophilic bacterial and fungal strains from bauxite residue based on their potential use in biotechnological applications.

Studying adaptation strategies of extremophilic organisms to extreme environments, such as the high pH, NaCl salt content, and the metal-rich conditions found in bauxite residues, may provide insights into their unique adaptations and survival strategies. Extremophiles can play a crucial role in bioremediation by utilising their metabolic capabilities to detoxify or remove pollutants from contaminated environments. Extremophiles often produce enzymes with exceptional stability and functionality under extreme conditions. These enzymes can have valuable industrial applications, such as in biotechnology processes that require extreme technology conditions. Our research also underlines the importance of harnessing extremophiles for biotechnology applications, as they can contribute to sustainable resource utilisation. This can include the development of bioprocesses for extracting valuable elements (e.g. rare earth elements) or converting waste materials into useful products.

In summary, the isolation of extremophilic bacterial and fungal strains from bauxite residue holds promise for various biotechnological applications, from bioremediation to the discovery of novel enzymes and bioactive compounds. It also contributes to our understanding of extremophiles and their adaptations, which has environmental and scientific research implications.

CRediT authorship contribution statement

Viktória Feigl: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Anna Medgyes-Horváth:** Formal analysis, Software, Visualization, Validation, Writing – original draft, Writing – review & editing. **András Kari:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft. **Ádám Török:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Nelli Bombolya:** Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Zsófia Berkl:** Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft. **Éva Farkas:** Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ildikó Fekete-Kertész:** Conceptualization, Data curation, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.btre.2023.e00825.

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