GENOMIC INSIGHTS OF AN ANDEAN MULTI-RESISTANT SOIL ACTINOBACTERIUM OF BIOTECHNOLOGICAL INTEREST

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23   **HIGHLIGHTS**

- 24   -   Arid Andean Soils are attractive sources of microbial strains useful in
- 25   biotechnological processes.
- 26   -   Physiological studies revealed the multi-resistant nature of the poly-extremophile
- 27   *Nesterenkonia sp. Act20*.
- 28   -   Act20's genome analysis showed a complete set of genes coding for proteins
- 29   involved in resistance to multiple stresses, including extremoenzymes and
- 30   extremolytes.

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33 ABSTRACT

34 Central Andean Ecosystems (between 2000 and 6000 masl) are typical arid to
 35 semiarid environments suffering from the highest total solar and UVB radiation on the
 36 planet but displaying numerous salt flats and shallow lakes. Isolated from these
 37 environments, Andean Microbial Communities (AME) of exceptional biodiversity
 38 endures multiple severe conditions. Also, the poly-extremophilic nature of AME's
 39 microbes indicates the potential for biotechnological applications. In this context, the
 40 presented study used genome mining and physiological characterization to reveal the
 41 multi-resistant profile of *Nesterenkonia* sp. Act20, an actinobacterium isolated from the
 42 soil surrounding Lake Socompa, Salta, Argentina (3570 m). UV-B, desiccation, and
 43 copper assays showed the strain's exceptional resistance to all these factors. Act20's
 44 genome presented coding sequences involving antibiotics, low temperatures, UV and
 45 arsenic resistance, nutrient limiting conditions, osmotic stress response, low
 46 atmospheric oxygen pressure, heavy metal stress, and resistance to fluoride and chlorite.
 47 Act20 can also synthesize proteins and natural products such as an insecticide, bacterial
 48 cellulose, ectoine, bacterial hemoglobin, and even antibiotics like colicin V and
 49 aurachin C. We also found numerous enzymes for animal and vegetal biomass
 50 degradation and application in other industrial processes .

51 The herein report shed light on the microbial adaptation to high-altitude
 52 environments, its possible extrapolation for studying other extreme environments of
 53 relevance, and its application to industrial and biotechnological processes.

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57 INTRODUCTION

58 Central Andean Ecosystems (between 2000 and 6000 masl) are typical arid to
59 semiarid environments suffering from the highest total solar and UVB radiation on the
60 planet, displaying numerous salt flats and shallow lakes (Albarracín et al., 2016, 2015).
61 Spanning from the Atacama Desert in Chile, through the Argentinean and Bolivian
62 Puna up to the Peruvian Andes, these ecosystems experience a wide daily temperature
63 range, high salinity (up to 30%), scarce nutrient availability and high concentration of
64 heavy metals and metalloids, especially arsenic (Albarracín et al., 2016, 2015).

65 Despite these conditions, Andean Microbial Communities (AME) prove
66 exceptional biodiversity and diverse strategies for enduring these severe conditions
67 (Albarracín et al., 2016, 2015; Solon et al., 2018). Likewise, the importance of
68 exploiting AME poly-extremophiles' full potential in terms of their biotechnological
69 applications was highlighted (Albarracín and Farías, 2012). Examples are the production
70 of waxes and fatty acids for biodiesel (Bequer Urbano et al., 2013) or compatible
71 solutes, antioxidants, pigments, or enzymes for the pharmaceutical industry (Farias et
72 al., 2011). Current projects heading this way have yielded detailed molecular
73 information and functional proof on novel extremoenzymes: i.e., photolyase of
74 *Acinetobacter* sp. Ver3 (Albarracín et al., 2014), an arsenical resistance efflux pump,
75 and a green tuned microbial rhodopsin (Albarracín et al., 2015) in *Exiguobacterium* sp.
76 S17 (Ordoñez et al., 2015).

77 *Actinobacteria* are high GC (50-71 %), Gram-positive microbes found in both
78 terrestrial and aquatic ecosystems (Albarracín et al., 2005; Montalvo et al., 2005). Being
79 the soil microbiota's main component (Schrempf, 2013), Actinobacteria exhibit various
80 morphologies (Ventura et al., 2007), physiological and metabolic properties, and
81 includes many species, which are useful in biotechnology (Kurtböke, 2003). Previous

work using dependent and independent culture techniques revealed that *Actinobacteria* is one of the predominant taxonomical groups among the AME's microbial communities (Dib et al., 2008, 2009; Rasuk et al., 2017). Moreover, AME's *Actinobacteria* have been demonstrated to carry giant linear plasmids that may involve the community's spread of resistance traits (Dib et al., 2010). AME's actinobacteria also showed their potential for producing secondary metabolites useful for the pharmacy industry. Wichner et al. (2017) reported that the extremotolerant isolate, *Lentzea* sp H45, synthesized new monoene, and diene glycosides. These natural products called lentzeosides A-F possess inhibitory activity against HIV integrase, a key enzyme for recombining the HIV genome into the host genome. Schulz et al. (2011) evidenced the production of bioactive compounds called abenquines (simple aminobenzoquinones substituted by different amino acids) by *Streptomyces* sp. DB634. Abenquines showed moderate inhibitory activity against phosphodiesterase type 4 (PDE4b) while proved useful for treating inflammatory diseases. Moreover, *Streptomyces* C38 produces 22-membered macrolactonic antibiotics atacamycin A-C, also considered drugs for treating inflammatory diseases by inhibiting PDE4b and antitumor by acting against tumor cell lines (Nachtigall et al., 2011).

Nesterenkonia is a particular genus (Stackebrandt et al., 1995) with most representatives isolated from hypersaline or alkaline environments such as saline soil, solar salt, seafood, soda lake, or alkaline wastewater. Halophiles are exploitable microorganisms for bioprocesses (Fu et al., 2014; Liu et al., 2019; Yin et al., 2014) (Yue et al., 2014). For example, *Nesterenkonia* MSA 31 isolated from a marine sponge *Fasciospongia cavernosa* produces a halo-alkali and thermal tolerant biosurfactant useful as an emulsifier stabilizing agent in the food industry (Kiran et al., 2017). Furthermore, *Nesterenkonia* sp. strain F isolated from Aran-Bidgol Lake (Iran) can

107 produce acetone, butanol, ethanol, acetic, and butyric acids under aerobic and anaerobic
108 conditions (Amiri et al., 2016). Also, *Nesterenkonia lacusekhoensis* *EMLA3* degrades
109 reactive violet 1 (RV1), a toxic azo dye, under conditions of high pH and in the
110 presence of a high concentration of NaCl, both of which generally inhibit microbial
111 treatment process (Prabhakar et al., 2019). Thus, *Nesterenkonia* strains are attractive
112 microbes in the search for biotechnological resources.

113 In a previous screening for extremophilic *Actinobacteria* from Puna arid alkaline
114 soil, the dark yellow-pigmented *Nesterenkonia* sp. Act20 strain was identified (Rasuk et
115 al., 2017). Act20 grew at a high concentration of NaCl (25%), Na₂CO₃ (5mM), and
116 arsenic (up to 200mM arsenate), in a wide range of pH 5-12, with optimal growth at
117 alkaline pH (Rasuk et al., 2017). In subsequent works, our group evidenced that
118 *Nesterenkonia* sp Act20 had a high tolerance to UV-B (up to 100 kJ/m²) due to an
119 integrated response to radiation called UV-resistome (Portero et al., 2019).

120 The following work aims to test the multi-resistance of the Act20 strain
121 combining phenotypic profiling with in-depth genomic analysis. Also, we highlight the
122 potential for biotechnological use of extremozymes and extremolytes coded in its
123 genome.

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125 1. MATERIALS AND METHODS

126 **2.1. Strains and culture conditions.** UV-resistant strain Act20 used in this study was
127 previously isolated from soil around Lake Socompa (3,570 m) at the Andean Puna in
128 Argentina (Albarracín et al., 2016, 2015) and belongs to the LIMLA-PROIMI
129 Extremophilic Strain Collection. Bacterial strain *Nesterenkonia halotolerans* DSM
130 15474 belongs to DSMZ Bacterial Culture Collection, and we used it as a control
131 following previously reported works (Rasuk et al., 2017). Both strains were grown in an

132 "H" medium (a medium modified for halophiles, containing NaCl 15 g L⁻¹, KCl 3 g L⁻¹,
133 MgSO₄ 5 g L⁻¹, sodium citrate 3 g L⁻¹) added with 2% agar when applicable.

134 **2.2. Multi-resistance assays.** The resistance of strain Act20 cells to diverse physical
135 and chemical stresses was tested when exposed to increasing concentrations of copper,
136 high UV doses, and desiccation treatments. For studying the response to desiccation,
137 cells were first grown aerobically at 30 °C overnight in a 10 ml nutrient broth medium
138 on a rotary shaker. Cells were harvested, washed once with sterile NaCl solution (0.85
139 %, w/v), and resuspended to reach an OD_{600nm} of 2 (±0.1) in NaCl solution.
140 Approximately 20 aliquots (100 µl each) from this cell suspension were spotted onto
141 0.45-µm filters (Sartorius, Göttingen, Germany). These filters were placed onto agar
142 medium H plates and incubated at 30 °C for five days. The colonies were then let dry by
143 incubation in empty sterile dishes at 25 °C and 18% of relative humidity for 50 days,
144 and the viable count (CFU) was assessed at different times. The filters on which the
145 strains were grown were added to sterile microcentrifuge tubes. The cells from each
146 filter were resuspended separately, and the CFU was determined before and after
147 desiccation treatment. The tolerance to desiccation was determined in Act20 and *N.*
148 *halotolerans* DSM 15474 as control.

149 Resistance to UV irradiation and copper salts were tested by a quick qualitative
150 method. For the copper resistance profile assays, aliquots of 5 µL of an overnight
151 (OD₆₀₀≈0.6) culture were loaded onto H medium agar plates supplemented with 1, 2, or
152 3 mM CuSO₄. The control cultures consisted of an "H" medium without copper
153 supplementation. Then they were incubated for 72 h at 30 °C under continuous PAR
154 luminosity conditions with an OSRAM 100 W lamp.

155 For the UV resistance assays, the cells were pre-culture on liquid medium H, and
156 once at OD_{600nm} = 0,6 collected for serial dilutions. Aliquots of 5 µL were then loaded

157 onto medium agar plates and immediately exposed to UV-B irradiation (Vilbert
158 Lourmat VL-4, the maximum intensity at 312 nm) 5 min (1,7 KJ m⁻²), 15 min (5,1 KJ m⁻²), and 30 min (10,4 KJ m⁻²). Then they were incubated for 72 h at 30 °C in the dark to
159 prevent photoreactivation. UV-B irradiance was quantified with a radiometer (Vilbert
160 Lourmat model VLX-3W) coupled with a UV-B sensor (Vilbert Lourmat model CX-
161 312). The minimal intensity measured was 5,21 W m⁻², and maximal power was 5,4 W
162 m⁻². Controls of unexposed samples were run simultaneously in the dark.

164 Microbial growth was recorded with tree signs (+++) when similar to the growth
165 in controls, two signs (++) when it was slightly different from the growth in the
166 controls, one sign (1 pts) when growth was low (isolated colonies), and no sign when it
167 was no growth at all. Parallel assays were performed for *Nesterenkonia halotolerans*
168 DSMZ 15474 to compare resistance profiles.

169 **2.3. Microscopic observation and ultrastructural characterization of Act20 cells.**

170 These assays were designed to evaluate the morphology and ultrastructure of Act20 in
171 challenging conditions similar to those present in their original environment, i.e., under
172 high UV irradiation and chemical (copper) stress. The selected strains were grown in H
173 medium at 30°C with shaking (180 rpm). Cells in the mid-log phase of growth were
174 harvested by centrifugation (5000 rpm for 10 min). Pellets were washed twice in 0.9 %
175 NaCl and were kept under starvation conditions for 18 h at 4 °C in the same solution. 20
176 ml of cell suspension was transferred to a sterile plate and were exposed to UV-B
177 irradiation at different times, as indicated before. Copper-challenged cultures were
178 likewise obtained by growing the cells in H medium with and without 3 mM Cu.

179 For scanning electron microscopy (SEM) and transmission electron microscopy
180 (TEM), 100 µL aliquots were collected for each different treatment and centrifuged

181 (5,000 rpm for 10 min) to remove the supernatant. The pellets were immediately fixed
182 with Karnovsky's fixative (a mixture of 2,66% paraformaldehyde and 1,66%
183 glutaraldehyde) in a 0.1 M phosphate buffer pH 7.3, for 48 h at 4°C. For SEM, the cells
184 were processed according to previously optimized methods (Zannier et al., 2019).
185 Briefly, aliquots of 50 µl of samples fixed were placed in coverslips for electron
186 microscopy and kept for three hours at room temperature. The samples were then
187 dehydrated in graded ethanol (30%, 50%, 70%, 90%, and 100%) for 10 min each and
188 finally maintained in acetone 100% for 40 min. The dehydration was completed with
189 the critical drying point (Denton Vacuum model DCP-1), in which acetone was
190 exchanged by liquid CO₂. Then, samples were mounted on stubs and covered by gold
191 (Ion Sputter Marca JEOL model JFC-1100) and observed under a Zeiss Supra 55VP
192 (Carl Zeiss NTS GmbH, Germany) scanning electron microscope belonging to the
193 Electron Microscopy Core Facility (CIME). For TEM, the protocol from Albarracín et
194 al. (2008) was followed. After fixation, samples were washed twice in 0.1 M phosphate
195 buffer, pH 7.3 (5000 rpm for 10 min), and embedded in agar (Bozzola, 2007). Agar
196 pellets were post-fixed in 1% osmium tetroxide in phosphate buffer, pH 7.3, overnight
197 at 4°C. After washing with the same buffer, and were stained in 2% uranyl acetate
198 solution for 30 min at room temperature. The samples were dehydrated with ethanol
199 solutions increasing concentrations (70%, 90%, and 100%) for 15 min each and finally
200 maintained in acetone 100% for 30 min. After that, the infiltration and embedding in an
201 acetone-SPURR resin sequence were carried out, followed by polymerization at 60°C
202 for 24 h. Ultrathin sections were cut using a diamond knife on a manual ultramicrotome
203 (Sorvall Porter-Blum Ultramicrotome MT-1). Bacteria were examined using a Zeiss
204 LIBRA 120 (Carl Zeiss AG, Alemania) transmission electron microscope at 80 kV,
205 belonging to the Electron Microscopy Core Facility (CIME-CONICET-UNT).

206 **2.4. Genome sequencing, assembly, and gap closure.** Genomic DNA from
 207 *Nesterenkonia* sp. Act20 strain was purified from cells grown on LB broth for 72 h at
 208 30° C and harvested by centrifugation (3,000 g for 10 min at 4°C). Pellets were washed
 209 twice with distilled water. We extracted total genomic DNA with the DNeasy Blood
 210 and Tissue Kit (Qiagen) following the manufacturer's recommendations. Whole-genome
 211 shotgun pyrosequencing was achieved using a 454 preparation kit (Roche Applied
 212 Sciences, Indianapolis, IN, USA) and sequenced with a GS-FLX using Titanium
 213 chemistry (454 Life Sciences, Roche Applied Sciences). The 454 reads were assembled
 214 with Newbler Assembler software, v. 2.5.3, with –URT option. Extra-assembling
 215 programs were run: MIRA v. 3.4.0 and Celera Assembler, v. 6.1. The different
 216 assemblages were fused using MINIMUS 2 Pipeline from the AMOS Package. The
 217 merged assembly was used as a guide for designing the primers, which were, in turn,
 218 used to confirm contig joints and close gaps. The overall sequence coverage was 37X;
 219 This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under
 220 the accession JADPQH000000000. The version described in this paper is version
 221 JADPQH010000000.

222 **2.5. Genome analyses.** Genome annotation was implemented using PROKKA
 223 (Seemann, 2014) with a custom expanded protein database, which includes Swiss-Prot,
 224 TrEMBL, Pfam, SUPERFAMILY, TIGRFAM, and a genus-specific database built from
 225 GenBank files downloaded from the NCBI database. We processed the set of annotated
 226 FASTA files belonging to genomes with Proteinortho, which detects orthologous genes
 227 within different species. For doing so, the software compares similarities of given gene
 228 sequences and clusters them to find significant groups (Lechner et al., 2011).

229 **2.6. Phylogenetic analysis.** The sequence-based taxonomic analysis was performed
 230 using both 16S rDNA and whole-genome comparisons. Sequences from genomes and

16S rDNA were obtained from the NCBI database (Assembly and RefSeq), and their characteristics resumed in Supplementary Table S1. Some 16S rDNA were retrieved from genome sequences to link both types of analysis. Sequences from 16S rDNA were aligned with Silva Incremental Aligner (Pruesse et al., 2012), and the phylogenetic tree was created with Fasttree 2.1.7 (Price et al., 2010) with the Maximum Likelihood method using Generalized Time-Reversible (GTR) model. Subsequent processing and visualization of the tree were performed with iTOL (Letunic and Bork, 2016). Genome-wide nucleotide identity trends were explored in the genome dataset by estimating all-against-all pairwise Average Nucleotide Identity (ANI). We utilized the ANIm approach that uses MUMmer (NUCmer) to align the input sequences as implemented in pyANI (Kurtz et al., 2004). The average between any given pair was used as the final value. Heat maps were generated using the heatmap V 1.0.12 R package (Kolde, 2019).

243

2. RESULTS

3.1. Taxonomic Affiliation of *Nesterenkonia* sp. Act20. *Nesterenkonia* sp. Act 20 was first isolated by Rasuk et al. (2017) and initially assigned to the genus by partial 16S rDNA sequencing. This work compared the full 16S rDNA sequence from Act20 with related strains, resulting in 98.2 % identity with *N. sandarakina* YIM 70009 and 97.4 % identity with both *N. jeotgali* JG-241 and *N. halotolerans* YIM70084. The phylogenetic tree based on this marker also suggests a close relationship of Act20 with the above mentioned plus *N. sp.* AN1, *N. aurantiaca* strains, *N. sandarakina*, and *N. Lutea*, clustering together in a significant clade (Fig. 1A). The whole-genome analysis using the ANI method shows a similar pattern of relationship (Fig. 1B). In this analysis, *N. sp.* Act 20, *N. aurantiaca* DSM 27373, *N. sp.* AN1, *N. jeotgali* CD087, and *N. sandarakina* CG 35 cluster with significance, and Act20 have a lower percent average nucleotide

256 difference (defined as 100% – ANI) value than the proposed 95% threshold (Richter
257 and Rosselló-Móra, 2009), suggesting that it could be a novel species.

258 **3.2. Multi-Resistance Profile of Act20.** Resistance to desiccation, UV, and copper was
259 uncovered in *Nesterenkonia* sp. Act20 and compared with the closest relative, *N.*
260 *halotolerans* (NH). A summary of this multi-resistance is presented in Table 1.
261 Tolerance to desiccation was tested every seven days for seven weeks. Act20
262 maintained its population in the same order of magnitude for 14 days, but from the third
263 week on, it showed null development (Fig. S1). In turn, NH decreased its population in
264 one order of magnitude after 7 and 14 days of treatment while it did not survive beyond
265 14 days of continuous drying conditions.

266 Tolerance to UV radiation was tested by placing culture serial dilutions drops of
267 the studied strains on "H" agar plates and exposing them to UV source as described
268 previously. A similar procedure was tried for testing resistance profiles to copper salts
269 in media amended with 1, 2, and 3 mM of copper. Our results showed that Act20 was
270 much more resistant to radiation than the selected control strain (Fig. S2), growing even
271 after a dose of 10,4 KJ m⁻² of UV-B radiation. In turn, the copper resistance profile was
272 similarly high for both strains; Act20 and NH developed quite well even at the
273 maximum concentration tested (3 mM).

274 **3.3. General Genomic Features.** The assembly process led to two scaffolds: the largest
275 with 2,092,188 bp in length and G + C content of 65.98%, and the second scaffold with
276 836,993 bp and G + C content of 65.82%. As a whole, the genome of *Nesterenkonia* sp
277 Act20 consists of 2,930,097 bp, with a GC content of 65.9%. PROKKA annotation
278 shows 2,672 coding sequences, including 2,377 annotated genes and 58 RNAs. Act20
279 genomics features were compared to the other fifteen *Nesterenkonia* genomes available
280 in the NCBI assembly database (Table S7). Supplementary Table S2 shows the

281 annotated genes of Act20, their sequence length, and functions assigned by homology,
282 as well as the KO identifiers linked for some of these genes. A summary of the most
283 relevant annotated functions can be found in Supplementary Tables S3 and S4.

284 **3.4. Genome traits of Act20 multi-resistance phenotype.** Genome inspection
285 indicated genetic determinants coding for systems potentially involved in the high
286 resistance profile observed in herein described and previous lab assays (Rasuk et al.,
287 2017). The genome showed pathways associated with osmotic and oxidative stress
288 response, low temperature, starvation response, and low oxygen conditions,
289 unmistakable evidence of this microbe adaptation to its extreme and changing habitat. It
290 also has traits related to resistance to heavy metals (mainly copper and mercury),
291 antibiotics (mainly beta-lactams and vancomycin), arsenic, fluoride, and chlorite (Table
292 S3, S4).

293 Both, the Prokka and RAST annotations account for several genes for
294 resistance/tolerance to copper (Table 2). Among the direct mechanisms of resilience to
295 copper described by Giachino and Waldron (2020) in Act20 is worth to mention the *cop*
296 family, implicated in copper homeostasis through the capture and expulsion of Cu[I]
297 ions from the cytosol to the periplasm. This mechanism may be complemented by the
298 action of a copper oxidase necessary for converting Cu [I] to Cu [II], which is more
299 biologically inert and tend to remain in the periplasm. It is also interesting the presence
300 of a gene matching a recently discovered family of copper-binding proteins involved in
301 cytosolic copper storage (Dennison et al., 2018). On the other hand, the indirect
302 mechanisms would involve the participation of genes whose actions compensate for
303 specific damage caused by toxic copper. This is the case for the specific DsbD oxidase
304 which rearranges misfolded peptides, and the Fur master regulator of iron metabolism

305 that counteracts the constant scavenging of iron cofactors from enzymes, which in turn
306 can generate ROS (Giachino and Waldron, 2020).

307 Previous assays carried out on Act20 revealed its ability to grow in alkaline
308 media (up to pH 12) and at high NaCl (25%) and Na₂CO₃ (5 mM) concentrations
309 (Rasuk et al., 2017). In this work, we also verify its desiccation tolerance, a phenotype
310 that is supported by a vast repertoire of genes (Table 3). This ability may be explained
311 by the presence of transporters for the uptake of a diverse organic osmoprotectant such
312 as glycine betaine, proline betaine, glycerol, choline, and trehalose. Genes for the
313 synthesis of glycine betaine and the complete set of genes for ectoine were also detected
314 (Table 3, Fig. S3). These compounds counteract the environment's high osmolality
315 avoiding a rapid efflux of water from the cell and, consequently, the loss of turgor. Low
316 turgor starts a rapid influx/synthesis of these osmo-protectants that complement another
317 inorganic compounds (Lucht and Bremer, 1994; Nagata and Wang, 2001; Styrvold and
318 Strom, 1991; Wood, 1988). Other genes, like OsmC and MdoB, are involved in coping
319 the osmotic stress in peculiar ways (Atichartpongkul et al., 2001), specifically MdoB,
320 whose product requires periplasm and outer membrane facilitate its effect (Sleator and
321 Hill, 2002), both structures not normally present in Gram-positive bacteria including
322 Act20.

323 The genomic profile of ABC transporters and the two-component system is
324 also a reflection of the original environment. The set of proteins to sense phosphate
325 limitation and subsequent phosphorus incorporation (in the form of phosphates and
326 phosphonates) are evident (Fig. S4, S5). A similar is noticed for osmotic stress. Other
327 environmental sensing types include oxygen limitation, low temperature, cell envelope
328 stress, cell wall stress, and antibiotics. Transporters for iron and various organic

329 compounds such as carbohydrates (mostly of plant origin), nucleosides, amino acids,
330 and oligopeptides were also reported.

331 To discover unique functional traits of Act20, we searched the orthologous
332 sequences for public available *Nesterenkonia* genomes. The set of annotated FASTA
333 files belonging to genomes were processed through Proteinortho, which detect
334 orthologous genes within different species. The proteins of Act20 that did not match
335 any ortholog were analyzed one by one to verify if the annotated function they encode is
336 unique for Act20 and is not present in other representatives of the genera. Table 4
337 shows the exclusive traits of Act20, most of them related to its extreme natural
338 environments such as bacterial persistence, bacterial cell envelope stress response, and
339 resistance to osmotic stress, desiccation, and phosphate starvation. Degradative
340 enzymes 6-deoxy-6-sulfogluconolactonase and α -xylosidase take part in the
341 decomposition of prototrophic biomass present in the soil, from which Act20 could take
342 advantage. The remaining functions were characterized only at the protein domain level,
343 like ethyl tert-butyl ether degradation, second messenger's sensors, and cell wall
344 binding.

345 **3.5. UV-Resistome of Act20.** The high UV-resistance profile of Act20 calls for more
346 in-depth characterization and points out the existence of integrated physiological and
347 molecular mechanisms triggered by ultraviolet light exposure. We named this system
348 "UV-resistome" as described before for other AME poly-extremophiles (Kurth et al.,
349 2015; Portero et al., 2019). Ideally, the UV-resistome depends on expressing a diverse
350 set of genes devoted to evading or repairing the damage provoked directly or indirectly.
351 Ideally, it encompasses the following subsystems: (1) UV sensing and effective
352 response regulators; (2) avoidance and shielding strategies; (3) damage tolerance and
353 oxidative stress response; and (4) DNA damage repair. Therefore, we screened genes

354 associated with each of these UV-B resistome subsystems for all available genomes of
355 the *Nesterenkonia* genus. This approach unveiled the relative genomic potential of
356 Act20 to defend itself from UV-B radiation, a strain that naturally endures the highest
357 irradiation on the planet. Unlike previous works, we studied the UV-resistance
358 integrally and included genes that could have the potential to generate a UV evasion
359 response or could lessen the negative impact the light, such as motility genes, pilus, and
360 gas vesicles (Damerval et al., 1991).

361 Table S5 details the UV-resistome for every strain and clusters it by subsystems.
362 The collections summarized all types of damage repair (base excision repair, nucleotide
363 excision repair, mismatch repair, homologous repair, direct repair, homologous repair,
364 direct repair, translesion DNA synthesis factors, and SOS response factors) explicitly,
365 oxidative stress response and UV avoidance/protection mechanisms (synthesis of
366 photoprotective pigments, and genes for flagellum, pilus, gas vesicles, and swarming
367 motility; Fig. 2). The bars are sorted from top to bottom, taking into account the number
368 of different genes for each subsystem. Act20 is positioned at the top with the most
369 diverse and complete UV-resistome, with 114 genes, followed by NBAIMH1 with 107
370 genes, which also belongs to an extreme altitude environment. Interestingly, other
371 strains from harsh environments with expected high solar radiation, such as AN1 and
372 M8 with 85 and 83 genes, respectively, also have robust UV-resistomes. On the other
373 hand, strains belonging to environments with little or null exposure to solar radiation
374 present less diverse and fewer genes: i.e., *N. alba*. DSM19423, GY074, and RB2 with
375 56, 63, and 65 genes, respectively.

376 **3.5. Ultrastructural changes in UV-challenged Act20 cells.** The morphology and
377 ultrastructure of the actinobacterium *Nesterenkonia* sp. Act20 was observed under UV
378 challenging and non-challenging conditions to determine their behavior in their original

environment. *Nesterenkonia halotolerans* DSM 15474 (NH) was used for comparison. Under SEM, in normal conditions, Act20 and DSM 15474 cells appear as irregular coccoids or short rod-shaped. Their size varied between 0.41-0.43 x 0.6-0.82 μm for Act20 and 0.41-0.38 x 0.71-0.51 for NH. The surface was smooth without evidence of cell wall rupture (Fig. 3A, 3E). In contrast, after exposure to UV both strains exhibited morphological alterations, which were consistently more severe in NH cells.

As the dose increases, Act20 morphology changed as individuals became longer and sometimes wide, probably due to cell division's interruption, causing a complete deformation in the cell (see black arrows in Fig. 3C, 3D). Furthermore, the cells' surface appeared with shrinkage signs (see white arrows in Fig. 3C). In NH, fibrillar structures were observed only on the surface of cells treated for 5 and 15 min with RUV (Fig. 3F, 3G). We found that at the dose of 0,51 Jls/cm² (15 min), the pili thickened, and in some sections, they broke or disintegrated (see Fig. 3G, white arrows). At 30 min of exposure (Fig. 3H), cell aggregates were observed in which bacterial cells adhered to one another by self-produced extracellular polymeric (EPS) substances. The surface irregularity was also found, indicating the wall cell rupture and degradation, probably causing cell lysis (Fig. 3H, black arrow).

TEM images for both strains were likewise obtained (Fig. 4). EM analysis revealed the typical Gram-positive bacteria structure, an intense electron opaque inner layer corresponding to the cytoplasmic membrane (CM) and a less electron opaque outer layer or cell wall (CW).

Ultrastructural changes were observed in the cells after exposure to UV radiation, especially at the structural membrane level, acquiring a certain degree of disorganization compared to the untreated strains (controls). A general observation in

both UV-treated bacteria was that numerous cells contained septa (S), compared to control samples. Radiation stress probably caused the cell division process to stop at this point without completing cell separation, whereas in control cells, the division was normal.

Furthermore, variations in the cell envelope thickness were frequently observed (see black arrows in Fig. 4). During the highest doses of UVR (15 and 30 min exposure), the different cytoplasmic structures were visualized by electron density variations. In Act20, polyphosphate-like granules (G) were visible, appearing as electron-dense aggregates, scattered throughout the cytoplasm but surrounding the nucleoid (N), with diameters varying between 0.2-0.3 μm (Fig. 4C, 4D); as well as mesosome-like structures (m) formed from projections of the cytoplasmic membrane (Fig. 4D). Also, the interaction between neighboring cells could be observed (see white arrows in Fig. 4D). In NH, the damage caused by UV at the cytoplasmic membrane level was more pronounced. The heterogeneous and disorderly appearance (see grey arrows in Fig. 4G, 4H) could result from loss of membrane integrity, leading to a malfunction of the permeability barrier and inducing cell lysis. Mesosome-like structures (m) (Fig. 4G, 4H) are frequent, and the interaction between bacteria mediated for pili or EPS is a common feature too (see white arrows).

3.5. Genome insights in Act20 biotechnological potential. Act20 is a halophile with enormous biotechnological potential, as it encodes haloenzymes and proteins with current applications in the food industry, waste treatment, medicine, cosmetics, biocontrol, pharmacology, paper industry, bioremediation, fuel, and chemical industry (Table 5).

Act20 could degrade wasted vegetal biomass, especially lignocellulose derivatives, as it encodes α -xylosidase, xylanases, xylose isomerases, and an α -

arabinofuranosidase. Also has a chitinase, which confers the potential to degrade wasted animal biomass. The genome of Act20 also reports the possibility of producing some exciting compounds of interest; these include bacterial cellulose, a compound with an emerging number of applications such as nanoparticle science and regenerative medicine (Gullo et al., 2018); bacterial hemoglobin, which was previously showed to enhance bioproduction upon low oxygen conditions (Frey and Kallio, 2003), promising antibiotics like colicin and aurachin (Gérard et al., 2005; Gerhard Hofle et al., 1987; Mousa et al., 2016; Oettmeier et al., 1994), and the protein Cry26Aa with high insecticide properties (Wojciechowska et al., 1999). Another relevant enzyme provided by the Act20 genome is the α -sialidase, which has been proven to be useful in synthesizing sialylated glycoproteins (Kim et al., 2011) highly suitable for pharmacology (Varki, 2008).

440

441 **3. DISCUSSION**

Nesterenkonia sp. Act20 is an actinobacterium isolated from an Andean soil in the Puna region, one of the most extreme environments on the planet, which even NASA has used to test microbes and equipment for spatial explorations (Cabrol et al., 2007; Cockell et al., 2019). Although the polyextremophilic nature of Act20 was preliminary explored before, its genome's functional characteristics have remained uncovered. In this work, we revealed the genomic basis of the multi-resistance phenotype of strain Act20, especially towards UV radiation, copper and desiccation. Its potential on the production of enzymes and compounds useful for biotechnology was likewise explored.

The 'extreme' environmental conditions suffered in its original environment –arid soil at 3,600 m- challenged Act20 to evolve mechanisms to tolerate a wide range

of chemical and physical stresses. They include strong fluctuations in daily temperature, hypersalinity, alkaline pH, high levels of UV radiation, a low nutrient availability, desiccation, and high concentrations of heavy metals and metalloids, especially arsenic (Albarracín et al., 2016, 2015; Dib et al., 2008, 2009; Farías et al., 2009; Fernández Zenoff et al., 2006; Ordoñez et al., 2009). *Nesterenkonia* strains were frequently isolated from environmental niches, including other saline locations (Amiri et al., 2016; Yoon et al., 2006). It was demonstrated that the ecology of these diverse habitats defines the genetic differentiation of *Nesterenkonia*. Such genetic differentiation seems to be a key feature in the genome of Act20 as it shares low sequence similarity with other strains of the set of genomes analyzed with ANI. The genomic and physiological particularities suggests Act20 may be a new species adapted to the HAAL environment; experiments heading this way are currently in progress in our lab (Fig. 1).

Act20's copper and desiccation resistance described before is coincident with several sequences found in its genome potentially involved in such resistance. It is noteworthy the abundance of direct and indirect mechanisms implicated in the copper resistance; some of them were well studied in Gram-negative bacteria and are performed by periplasmic proteins, which are unlike to exist in the Gram-positive Act20 which lacks outer membrane and periplasm (Giachino and Waldron, 2020). Likewise, the desiccation resistance relies on the production of a wide diversity of osmoprotectants that creates a hydrostatic force in the cytoplasm; the puzzling fact is that MdoB protein would provoke such hydrostatic pressure only in the periplasm. In both cases, it will be interesting to assess whether Act20 cell wall structure can accommodate a periplasmic-like space where the described gene products can function as proposed for Gram-negative bacteria, periplasmic space was reported for other Gram-positive bacteria (Matias and Beveridge, 2005; Zuber et al., 2006).

478   A particular cytosolic copper storage protein in the annotation analysis
479   called our attention. Its function and existence are controversial since a widely accepted
480   view is that bacteria have not evolved to use intracellular copper accumulation due to
481   potential toxicity associated with their metalation (Dennison et al., 2018). Note that this
482   protein was initially discovered in the Gram-negative methane-oxidizing bacterium
483   (methanotroph) *Methylosinus trichosporium* OB3b, which use large amounts of copper
484   to metabolize methane via the membrane-bound (particulate) methane monooxygenase
485   (pMMO) (Vita et al., 2015). Nevertheless, it is not clear the role of this copper storage
486   in Act20 which can otherwise work as an additional osmoprotectant. More research will
487   be needed to confirm the copper bioaccumulation ability of Act20, a mechanism useful
488   for the design of bioremediation processes and applicable to several pollutant activities
489   such as industry and mining.

490   The complete set of genes for ectoine synthetic cluster is present in Act20
491   genome (Fig. S3), conferring the strain an excellent potential for future biotechnological
492   applications. Ectoine is a water-binding zwitterionic amino acid derivative with
493   numerous biotechnological applications. It is a common component of cosmetic anti-
494   aging and moisturizing creams to improve skin resistance to surfactants in skin
495   cleansing solutions. It also alleviates skin inflammation, being currently recommended
496   to treat moderate atopic dermatitis (Bownik and Stępniewska, 2016). Furthermore, the
497   compound is useful in sunscreens as it strongly absorbs ultraviolet (UV) radiation and
498   protects DNA from breaking down in diverse cell types. Ectoine also has applications in
499   medicine: it can inhibit HIV replication and stabilize retroviral vectors for gene therapy
500   (Bownik and Stępniewska, 2016). The alleviation of certain kinds of inflammation
501   (colitis and neutrophilic lung inflammation) and allergic rhinitis were also reported,

502 even preventing the amyloid formation and delaying the onset and progression of
503 Alzheimer's disease (Bownik and Stępniewska, 2016).

504 Metabolism of arsenic, a toxic element that can limit or suppress bacterial
505 growth, is also possible for Act20. It has previously been demonstrated that Act20 can
506 tolerate arsenic in the form of As (V) (0-200 mM) (Rasuk et al., 2017). In this work, we
507 reported the presence of an arsenate reductase (not specified), an arsenate-myocthiol
508 transferase, an arsenic transporter (not specified), and seven regulators of the ArsR
509 family (Table S3). We propose that this resistance probably occurs by reducing As (V)
510 to As (III) with a myocthiol-dependent arsenate reductase and subsequent efflux of As
511 (III) from the cell using specific transporters (Ordóñez et al., 2009). Thus, the genome
512 of Act20 joins others already sequenced from HAAL extremophilic prokaryotes
513 (Burguener et al., 2014; Farias et al., 2011; Ordoñez et al., 2015, 2013), being the first
514 of the *Nesterenkonion* genus reported for an environment with a high concentration of
515 arsenic, and the first HAAL genome reporting the reduction of As (V) to As (III)
516 through the myocthiol/thioredoxin redox pathway.

517 The genome of Act20 also seems to be optimized to cope with low nutrient
518 availability, particularly phosphorous, as genes for a phosphorous-specific two-
519 component system and transporters are well represented (Fig. S4, S5). The two-
520 component system detects low phosphorous availability and communicates the signal to
521 the cell, which expresses phosphate and phosphonate active transporters. Also, two
522 phosphate starvation inducible proteins were reported in the annotation, PhoH, and
523 SphX (Table 4, Table S3), being the latter unique of Act20 and no present in other
524 *Nesterenkonion*. The genomic data also reveals that Act20 could face carbon starvation
525 as its genome codifies active transporters to uptake several kinds of peptides,
526 nucleotides, amino acids, and carbohydrates from the environment (Table S3, Fig. S4).

527 *Nesterenkonia* sp. Act20 genome is the first one of this genus reported from
528 the highest UV irradiation environment on Earth: Puna-High Andes region; in
529 accordance, we found genetic traces for complex UV resistance mechanisms
530 comprehensively called as UV-resistome. The comparison of UV-genomic determinants
531 of Act20 with other *Nesterenkonia* genomes indicated indeed a more sophisticated UV-
532 resistome for the Socompa strain. It is then evident that environmental irradiation has a
533 notable impact on the genome of Act20, which has a higher quantity and diversity of
534 genes dedicated to UV resistance than other strains less irradiated. We also observed a
535 similar pattern for other *Nesterenkonia* from high altitude or expected high irradiated
536 environments (Fig. 2, Table S7). Interestingly, solar irradiation intensity selecting UV-
537 resistome gene abundance and diversity in aquatic microbiomes was also evidenced by
538 our group using a worldwide metagenomic analysis (Alonso-Reyes et al., 2020).

539 Among the subsystem of UV evasion/shielding is worth to note the genes
540 involved in the production of gas vesicles (gvpA, K, O and F). Certainly, the expression
541 of gas vesicles (Damerval et al., 1991; Englert et al., 1992; Pfeifer, 2012) along with
542 flagella, allow microbes to move up in the water column toward sunlight. However, in
543 many cases, these vesicles are also present in soil prokaryotes, even from high UV
544 radiation environments, thus suggesting new roles in radiation protection (Oren, 2012).
545 It has been speculated that they could change the cell position concerning the angle of
546 the light's incidence, changing its impact on the cell (Bolhuis et al., 2006; Oren, 2012).
547 Additionally, our current works on comparative proteomics reveal an increase in vesicle
548 proteins' expression in response to the UV increase (Zannier et al., in preparation). We
549 also include flagella, swarming motility proteins and the pili, whose ability to promote
550 bacterial aggregation or biofilm adhesion may protect the cells from the UV exposure
551 (Burdman et al., 2011; Ojanen-Reuhs et al., 1997).

Act20 genome codes resistance genes to other extreme factors: low temperatures, low atmospheric O₂ pressure, heavy metal and other toxic compounds stress. Many of these hard environmental factors added to the previously mentioned and geophysical characteristics of the sampling site resembles those present in Early's Earth atmosphere that witnessed the evolution of ancient microorganisms (Albarracín et al., 2015; Cabrol et al., 2007) (Fig. 5) (Cockell et al., 2000; Forni et al., 2015; Hecht et al., 2009; Karunatilake et al., 2007; Sforza et al., 2014; Wadsworth and Cockell, 2017; Yen et al., 2006) (<https://mars.nasa.gov/all-about-mars/facts/>). Thus, Act20 is an exciting model to study the mechanisms by which the extremophiles could have successfully faced the adverse conditions of the Earth's history, having clear implications on astrobiological projects (Hiscox and Thomas, 1995; Merino et al., 2019; Slotnick, 2000).

Act20 could also have a great biotechnological potential for producing and metabolizing compounds and enzymes of interest (Table 5). Bacterial cellulose, hemoglobin, antibiotics, and a potential insecticide are among the Act20 biosynthetic products. On the other hand, several genes for enzymes capable of degrade vegetal biomass were also detected. This feature is of importance for the biodegradation of lignocellulosic biomass of agro-industrial wastes which are produced in large amounts through agricultural and forestry practices, including the paper-and-pulp and timber industries. On the other hand, animal biomass that contains high proportions of shellfish, such as shrimp, crab, and krill, are suitable to be processed through chitinases, an enzyme that is also present in Act20. The seafood processing industry has raised serious concerns regarding disposal issues because of this waste's low biodegradation rate, which could be solved through an enzymatic approach.

576   The enzymes mentioned above could be used in industrial processes that
577 reproduce the microbe's original natural habitat's extreme conditions. Biocatalysts
578 isolated by these extremophiles are termed extremozymes and possess special salt
579 allowance, thermostability, and cold adaptivity. Extremozymes are very resistant to
580 severe conditions owing to their great versatility. As such, they represent new prospects
581 for biocatalysis and biotransformations and the development of the economy and unique
582 line of research through their application (Dumorné et al., 2017). Here, we also report
583 the genetic potential of Act20 to provide poly-extremozymes, which could combine
584 resistance to cold (-2 ° C - 20 ° C), high solar radiation, salinity (at least 1 M salt), and
585 high pH (>8). Enzymes of this halophilic microbe could provide great opportunities,
586 particularly for food, bioremediation, and pharmacy industries.

587

588 **CONCLUSION**

589   In this work, we have confirmed by lab assays the multi-resistance
590 phenotype of *Nesterenkonia* sp. Act20, a poly-extremophile originally isolated from
591 Puna arid soil surrounding Lake Socompa, in the Puna region, exposed to the highest
592 irradiated environment on Earth. Accordingly, its genome codes for a plethora of genes
593 that help counteract the ecological pressure of the hostile conditions face by the microbe
594 in its original environment: i.e. arsenic, nutrient limiting conditions, osmotic stress, UV
595 radiation, low temperatures, low atmospheric O₂ pressure, heavy metal and other toxic
596 elements stress.

597   As a novel extremophile, Act20 has the potential to produce compounds
598 (extremolytes and extremoenzymes) of interest with application in industrial processes
599 such as an insecticidal protein, bacterial cellulose, ectoine, colicin V, araucins,

600 chitinases and cellulases. In this way, Act20 becomes an exciting candidate for
601 additional studies of transcriptomics, proteomics (currently in progress), metabolomics,
602 as well as the expression and testing of biotech-competent enzymes. The herein report
603 shed light on the microbial adaptation to high-altitude environments, its possible
604 extrapolation for studying other extreme environments of relevance, and its application
605 to industrial and biotechnological processes.

606

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921

922 **Figure and table legends**

923 **Figure 1.** Phylogenetic analysis of *Nesterenkonia* strains. Strain *Nesterenkonia* sp.
924 *Act20* is in bold. (A) Maximum likelihood tree from the 16S rRNA gene. Black dots
925 indicate that the gene was retrieved from a sequenced genome. (B) Heatmap based on
926 whole-genome average nucleotide identity (ANI). *Act20* is distinct and inside the
927 significant cluster.

928

929 **Figure 2.** The stacked bar chart shows the count of genes for all UV-resistome
930 subcategories in colors, including the total count at the right end.

931

932

933 **Figure 3.** Scanning electron microscopy (SEM) micrographs of actinobacteria after
934 exposure to UV-B radiation. **(A-D)** *Nesterenkonia* sp. *Act20*. **(E-H)** *Nesterenkonia*
935 *halotolerans* DSM 15474. **(A, E)** Non-exposed growing bacterial cells (control). **(B, F)**
936 Growing bacterial cells exposed to 0,17 Jls/cm² of UV-B (5 min). **(C, G)** Growing
937 bacterial cells exposed to 0,51 Jls/cm² of UV-B (15 min). **(D, H)** Growing bacterial
938 cells exposed to 1,04 Jls/cm² of UV-B (30 min). Black arrows indicate changes
939 morphological, and white arrows indicate damage in cells (C) and pili deterioration (G).
940 Scale bar 200 nm (E, F, G, H), 100 nm (A, B, C, D, E, F, H).

941

942 **Figure 4.** Transmission electron microscopy (TEM) micrographs of actinobacteria cells
943 after exposure to UV-B radiation. **(A-D)** *Nesterenkonia* sp. *Act20*. **(E-H)** *Nesterenkonia*
944 *halotolerans* DSM 15474. **(A, E)** Non-exposed growing bacterial cells (control). **(B, F)**
945 Growing bacterial cells exposed to 0,17 Jls/cm² of UV-B (5 min). **(C, G)** Growing
946 bacterial cells exposed to 0,51 Jls/cm² of UV-B (15 min). **(D, H)** Growing bacterial

947 cells exposed to 1,04 Jls/cm² of UV-B (30 min). Scale bar 200 nm principal figure and
948 50 nm small box.

949 **Figure 5.** Comparison between the extreme conditions (relevant to Act20) of the Lake
950 Socompa, Mars surface, and the early Earth environments. The light brown box
951 represents the Act20 genome and its potential for the different resistances as described
952 inside.

953

954

955 **Table 1.** Multi resistance profile of Act20 strain and comparison with *Nesterenkonia*
956 halotolerans (NH)

957 **Table 2.** Group of genes potentially involved in the copper resistance reported for
958 Act20.

959 **Table 3.** Group of genes potentially involved in the desiccation resistance reported for
960 Act20.

961 **Table 4.** New functional traits (with no counterpart in other *Nesterenkonia*) of
962 *Nesterenkonia sp. Act 20* unveiled by genome functional annotation and ortholog
963 clustering.

964 **Table 5.** List of Act20's annotated enzymes and proteins with reported biotechnological
965 potential.

966 Tables

967 Table 1.

	Act20	NH
Desiccation Period		

7-days	+++	+
14-days	++	+
21-days	-	-
Copper Concentration		
1 mM	+++	+++
2 mM	++	++
3 mM	+	+
UV Dose		
1,7 Kj m⁻²	+++	++
5.1 Kj m⁻²	++	+
10.4 Kj m⁻²	+	+

968

969

970 Table 2

Copper tolerance/resistance trait	Function
Copper-translocating P-type ATPase	Probably similar to P-type ATPase CopA, it secretes Cu(I) from the cytoplasm to the periplasm to maintain copper homeostasis (Giachino and Waldron, 2020).
CopC domain protein	CopC is a bacterial blue copper protein that forms a dinuclear copper molecule. Along with CopA, CopC mediates copper export from the cytoplasm to the periplasm (Arnesano et al., 2003).

Putative CopD protein	Exact function not known. Involved in copper uptake in conjunction with CopC (Swiss)
CopZ	Chaperone that serves for the intracellular sequence from the cytosol to the periplasm (Giachino and
Copper oxidase	Probably a periplasmic copper oxidase-like C the cytosol by converting periplasmic Cu(I) to inert, making it a relatively “safe” species for c (Waldron, 2020).
DsbD domain-containing protein	DsbD (also known as CutA) oxidoreductase re incorrect disulfide bonds are inserted by toxic c
Four-helix bundle copper-binding protein	Cu(I) cytosolic storage. First found in meth amounts of copper to metabolize methane. Its what they are storing copper for remains unknown
Fur family ferric uptake transcriptional regulator	As toxic copper provokes mismetalation o regulator of iron metabolism is overexpressed i copper resistance even at low copper concentra

971

972 Table 3.

Desiccation tolerance/resistance trait	Function
SugA-SugB-SugC	ABC transporter complex particular for the Involved in the recycling of extracellular containing molecules (Kalscheuer et al., 2010)
Trehalase	Trehalose degradation. It may be nece

	intracellular trehalose (Carroll et al., 2007).
opuBC-OpuBB-OpuBA	High affinity multicomponent binding-prote osmoprotectant choline (Kappes et al., 1999)
ProX-ProW-ProV	ABC transporter complex involved in the betaine and proline betaine (Gul and Poolman
Choline dehydrogenase	Involved in the biosynthesis of the osmopr 1996).
Betaine-aldehyde dehydrogenase	Involved in the biosynthesis of the osmoproc reversible oxidation of betaine aldehyde to 1997).
HTH-type transcriptional regulator BetI	Repressor involved in the biosynthesis of t represses transcription of the choline trans involved in the synthesis of glycine betaine (
EctA-EctB-EctC-EctD	Group of genes involved in the biosynt (Reshetnikov et al., 2006).
Glycerol uptake facilitator protein	Transporter of the osmoprotectant glycerol (Hénin et al., 2008).
Phosphoglycerol transferase I MdoB	Participate in anionic polymers' biosynthe glucose units with an average charge of -5 potential across the outer membrane, resulti higher concentration in the periplasm than in to hydrostatic pressure in the periplasmic spa
OsmC	The osmotically inducible expression of the kind of role in the bacterial osmotic-stress res

974 Table 4.

Unique trait	Function
Protein SphX	High-affinity phosphate-binding protein inducible (Gobler et al., 2013; and Gobler, 2013)
Serine/threonine-protein kinase toxin HipA	Toxin that induces bacterial persistence and (Gobler et al., 2013).
6-deoxy-6-sulfogluconolactonase	Sulfoquinovose degradation (Felux et al., 2015)
Trehalase	Trehalose degradation (Carroll et al., 2007).
α-xylosidase	Catalyzes the liberation of alpha-xylose from tetrakisaccharides and xyloglucan oligosaccharides (Matsuzawa et al., 2007).
4-deoxy-L-threo-5-hexosulose-uronate ketol-isomerase	Plays a role in the catabolism of hexuronate and is likely substituting for the regular hexuronate isomerase. Expression is repressed in these conditions (Rochet et al., 2010).
Pleckstrin homology domain (bPH_1)	Appears to be involved in the bacterial cell cycle (Rochet et al., 2010).
SMODS-associated and fused to various effectors sensor (SAVED) domain	Senses nucleotide or nucleotide derivatives and various effectors deployed by a class of conflict systems. Involved in sensing of the nucleotide second messengers (Fischer et al., 2010).
WxL domain	Cell wall binding in gram-positive bacteria. Binds to peptidoglycan (Brinster et al., 2007).
EthD domain	Related to the EthD protein, which is involved in the degradation of ether (ETBE) (Chauvaux et al., 2001)
Coronavirus endopeptidase C30	Involved in viral polyprotein processing in replication (Cramer et al., 2015)

976 Table 5.

Trait	Biotechnological applications
Alpha-xylosidase	Although α -xylosidases are relevant for the degradation of xylan saccharides, only a few α -xylosidases have been characterized. Unclear aspects regarding the properties of the enzyme are still present (Matsuzawa et al., 2016). Before α -xylosidase can be used, it is necessary to study its properties, and N. Act 20 α -xylosidase is especially from the fact that N. Act 20 α -xylosidase is a
1,4-beta-xylanases	Xylanases are used in the paper industry to improve the bleaching agents (hydrogen peroxide, ozone, chlorine) and to reduce the hemicellulose, reducing the amounts of bleaching agents, reducing process cost and increasing its eco-friendliness. Xylanases are also used in the clarification of fruit juices and wines for improving the texture of bread. Furthermore, xylanases are used as food additives (Qeshmi et al., 2020).
Xylose isomerase	Usable in microbial cell factories or biorefineries for the production of fuels, chemicals, and other industrial derivatives.
Chitinase (glycosyl hydrolase family 18)	Chitinases are suitable for 1) waste treatment, 2) production of chitoooligosaccharides and GlcNAc (which are used in the production of single-cell proteins for usage as food additives), 3) post-harvest fungal pathogens, and 5) functional foods. Chitinase is used for example as a treatment for various fungal diseases.
Exo-alpha-sialidase	Synthesis of sialylated glycoproteins. Sialic acid is an important component of the half-life of glycoproteins in circulation. Thus, it is used in the synthesis of sialylated glycoproteins.

	monosaccharides such as galactose are recognized by various organs, and the glycoprotein is rapidly cleared from the body. This has practical relevance for pharmacology because many drugs (antibodies, cytokines, and hormones) are glycosylated (2008).
Alpha-L-arabinofuranosidase	Among the functions of microbial α-arabinofuranosidase are: 1) Increase digestibility in the animal feed industry, 2) improve texture, and delay staling of bread, 3) increase shelf life in the food industry, 4) serves in the clarification of juice, 5) reduce sugar content in bioethanol industry, 6) used in the production, antiglycemic agent production, 7) sweetener production, and mycobacterial diseases.
Cellulose synthase/poly-beta-1,6-N-acetylglucosamine synthase-like glycosyltransferase	These enzymes have critical roles in bacterial cellulose synthesis. Cellulose is a malleable material that makes of it of enormous importance in science, specially designing celluloses with tailored properties. Native BC does not require any purification and has a number of biomedical applications. Advancements in regenerative medicine led to his usage in artificial hemostatic materials. One of the most exciting applications is as wound healing scaffolds. BC is also applied in the cosmetic area. Regarding to the cosmetic area, BC is extensively used as a powder in facial scrubs in association with other ingredients. One application in cosmetic is the production of coatings with high light transmittance, and permeability to liquid. Cellulose from BC can be used also in drug delivery for
Cellulose biosynthesis protein BcsQ	
Cellulose synthase	

	key role also in the food industry being used as beverages (due its ability to acquire flavors additive, it is used worldwide for its gelling hydrogel-like texture could be a new material Finally, BC is also applicable to bioremediati mercury and arsenic has been proved (Gullo et
Colicin V production protein	Colicin V (colV) is a secreted peptide antibio reducing competition for nutrients (Gérard e production of colV by an Enterobacter sp. stra Fusarium wilt in finger millet (Mousa et al., 201
Aurachin C monooxygenase/isomerase	This enzyme catalyzes the initial step in con Aurachins are quinoline alkaloids that act as a They have been reported to exhibit antimicro positive bacteria (Gerhard Hofle et al., 1987; O
Protein Cry26Aa	Promotes colloidosmotic lysis by binding to t having the potential to be used as a pesticidal (
Hemoglobin	Heterologous expression of bacterial hemoglo beneficial for mycelium forming microorganis Streptomyces strains, Acremonium crysogenur It is known that oxygen transfer to the myceli critical when such microorganisms are grown i such situations, BHb expression seems to enha improved the bioremediation capacity of Burk rate of 2,4-DNT degradation, where oxygen degradation pathway. Moreover, heterologous

		oxygen limitation in a wide range of distin
		productivity (Frey and Kallio, 2003).
Ornithine cyclodeaminase		Can be used for biotechnological production
		overexpressed in <i>C. glutamicum</i> (Jensen and W
		It may be useful in radiopharmaceutical app
Molybdate-binding protein	ModA	bond-containing protein with increased affinity
(Molybdate/tungstate-binding protein ModA)		may be used to remove chromate, which is tox
		environmental aqueous solutions (Karpus et al.,

A **B**

A **E****B** **F****C** **G****D** **H**

A **E** **B** **F** **C** **G** **D** **H**

High UV
High arsenic content
Heavy metal content
Hypoxia
-20 °C to 20 °C
Hypersalinity
Low nutrient availability

Lake Socompa
(surrounding soil)

Martian surface

High UV
High heavy metal content
Perchlorates
Fluorine containing minerals
Hypersalinity
-140 °C to 30 °C

UV-resistome
Base Excision Repair
Nucleotide Excision Repair
Mismatch Repair
Homologous Repair
Direct Repair
Translesion DNA Synthesis Factors
Oxidative Stress
Ectoine + Carotenoids
Flagellum + Swarming Motility
Pilus IV
Gas Vesicle

Heavy metal resistance
Copper, cobalt, zinc, chromate, cadmium

Arsenic resistance
arsenate reductase
arsenate-mycotoxin transferase
arsenic transporter

Fluoride resistance
Resistance protein CrcB

Osmoprotection

Specific ABC transporters
Synthesis of osmoprotectants
Trehalase degradation

Nutrient starvation resistance

Phosphate and Phosphonate ABC transporters
Phosphate limitation Two-Component System
Phosphate starvation inducible proteins PhoH and SphX
Carbon starvation protein CstA

Oxygen uptake optimization
Hemoglobin

Cold resistance
Cold-shock proteins

Perchlorate respiration ?
Chlorite dismutase

Early Earth

High UV
Low nutrient availability
Arsenic cycling in oceans ? Sforma *et al.* 2014
Oxygen gradually increasing during proterozoic