The Biotechnological Potentials of Bacteria Isolated from Parsık Cave, Turkey

Measuring the enzyme profiles, antibiotic resistance and antimicrobial activity in bacteria

1. Introduction

Caves are dark environments with high humidity, low nutrients, stable temperature and high mineral diversity. They are natural geological formations constituting ecological niches for microorganisms (1). Each cave is singular in its physical, chemical, biological and ecological factors. These conditions contribute to the formation of unique microbial communities in every cave. Moreover, caves contain some unique microorganisms which lead to rock weathering process and biomineralisation by carrying out various enzymatic reactions as a result of their metabolism. These microorganisms play an important and major role in the formation of cave structures such as stalactites, stalagmites, cave pearls and curtains (2–5). Studies have shown that cave isolates have biotechnological and industrial applications such as microplastic degradation (6), biological treatment of metal contaminated soil and groundwater (7) and use in self-healing concrete (8).

The insufficient nutrient levels in caves stimulate competition among microorganisms by forcing them to develop survival strategies such as producing high amounts of exopolymeric substances, enzymes and antimicrobial metabolites. Hence, caves could be considered as incomparable environments for the discovery of new antibiotics and production of novel enzymes (9–11).

Since cave ecosystems have extraordinary environmental conditions, these ecosystems offer opportunities for microbiological studies. In this study, cultivable bacteria isolated from Parsik cave, Turkey, were investigated regarding enzyme profiles, antibiotic resistance and potential for production of antimicrobial agents. The metabolic properties of 321 bacterial isolates were determined. The most produced enzyme by the isolates was found to be tyrosine arylamidase. The enzymatic reactions of the bacteria showed that Parsık cave isolates have high aminopeptidase activity. The highest antibiotic resistance frequency of the isolates was 38.6% against ampicillin. While the isolates displayed variable inhibition rates against tested pathogenic microorganisms, they showed the highest inhibition against Candida albicans. The results show that the bacteria isolated from Parsık cave have potential for further studies related to biotechnological applications. The study findings contribute increased knowledge on metabolic peculiarities of bacteria isolated from cave ecosystems.
used in biotechnological and industrial fields such as biodegradation, recycling of waste (12), purification and dirt or waste-dissolving products. It is reported that enzymes from microorganisms isolated from cold cave or ocean environments offer economic benefits and contribute to energy conservation due to their activation at low temperatures (13, 14).

Apart from the importance of enzymes isolated from cave microorganisms, it is interesting to investigate the potential of producing new antimicrobial agents. Since the World Health Organization pointed out the need for new antibiotics because of increasing microbial resistance (15), studies in this field are multiplying and many cave isolates producing antimicrobial substances have been discovered. Cervimycin A, B, C and D from *Streptomyces tendae* strain HKI 0179 isolated from Grotta dei Cervi in Italy (16), Xiakemycin A from *Streptomyces* sp. CC8-201 isolated from Chongqing City karst soil in China (17), and Hypogeamicin A, B, C and D from *Nonomuraea specus* isolated from Hardin’s cave system in Tennessee, USA (18) were the first produced and purified bioactive substances from microorganisms of caves situated in different geographical regions.

Bacteria in environments far away from human influence are not expected to have antibiotic resistance. However, studies have shown that bacteria isolated from such environments do have antibiotic resistance. Some bacteria have resistance genes by which they can produce neutralising or detoxifying products which act against microorganisms in the same environment. This explains the imperative production of antibiotics in these bacteria. Since the resistance and antimicrobial biosynthesis genes are often linked and coregulated, antibiotic resistance in environmental bacteria remains a major indicator of antibiotic production, as is the case of bacteria isolated from soil (19, 20). Therefore, it is important to establish antibiotic resistance profiles as well as the antibacterial properties of bacteria.

This study has two main goals:
- Detection of enzyme profiles of the isolates and determination of isolates that have potential uses in biotechnology
- Investigation of antimicrobial agents and antibiotic resistance of cave bacteria.

2. Experimental

2.1 Studying Area and Sampling

Parsık cave is located in Izmit-Aksığın village (Global Positioning System (GPS) coordinates 40° 37’ 50.1060”N, 29° 57’ 56.5056”E), in the north-west of Turkey. It is a horizontal cave with a length of 778 m and a depth of 166 m. There is an intense water inlet in Parsık cave throughout four seasons. Samples were taken from water, soil and surface formations (‘moonmilk’) (Figure 1). The selected
sampling zones are the sole area away from the entrance area, trip and running water pathway. Although Parsık cave is not a show cave, it is open to cavers and researchers.

Surface formation samples were collected by sterile swabs under aseptic conditions and cultivated on starch casein agar (SCA), inorganic salt-starch agar (ISP4), soil extract agar (SEA) and Actinomycetes isolation agar (AIA-G) in duplicate for each region. Once the plates reached the laboratory, they were incubated aerobically for a period of 5–30 days at 20°C (21). All water and soil samples were taken in sterile sample containers.

2.2 Physicochemical Measurements of Sampling Areas

Humidity and temperature values of the sampling areas were measured by a portable temperature/humidity meter. In addition, the temperature, conductivity, amount of dissolved substances and pH values of the sampled water sources were measured during sampling and recorded by a HQ40D digital two channel multimeter (Hach Lange GmbH, Germany).

2.3 Total (Live/Dead) Bacteria Number

The redox dye 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC) was used together with the DNA-binding fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) to determine the total number of bacterial cells and the viable count of bacteria which actively respire. The concept is to distinguish between the metabolically active cells and the dead cells present in each of the water and soil samples. The experimentation procedure is the same as previously described by Güngör and Yurudu (22).

2.4 Enumeration and Isolation of Culturable Aerobic Heterotrophic Bacteria

1 l of water samples were condensed by using polyamide filters of 0.22 µm pore size. Filters were re-suspended in 20 ml of sterile physiological saline water. 1 g of the soil samples was homogenised in 9 ml of sterile physiological saline water. All samples were cultivated using the 10-fold serial dilution method. Diluted samples were cultured on tap water agar (TWA) and Reasoner’s 2A agar (R2A) for enumeration and isolation of bacteria from water and soil samples. In addition, bacterial isolation from soil samples was on SCA, ISP4, AIA-G, SEA and 1/2 tryptic soy agar (TSA) media, and that from water samples was on 1/2 TSA only. Plates were incubated aerobically for a period of 5–30 days at 20°C (21). At the end of incubation, plates which contained between 30 and 300 colonies were considered for both soil and water samples. Colonies which appeared different were selected for identification, then stored at −86°C for subsequent uses.

2.5 Identification of Cave Isolates and Their Enzymatic Reactions

Cave isolates were identified through biochemical tests performed in the VITEK® 2 system (bioMérieux SA, France). One of the three formats of this system is the VITEK® 2 Compact 30 which focuses mainly on the industrial microbiology-testing environment. Based on this industrial software, three reagent cards of VITEK® 2 Compact 30, named Gram-negative fermenting and non-fermenting bacilli (GN), Gram-positive cocci and non-spore-forming bacilli (GP) and Gram-positive spore-forming bacilli (BCL), were used to characterise the isolated bacteria following the procedure and data given by the system manufacturers. Reagent cards are based on established biochemical methods and developed substrates (23). The results of biochemical reactions were interpreted to establish enzymatic profiles of isolates.

2.6 Ability of Cave Bacteria to Produce Antimicrobial Materials

The ability of Bacilli or Actinobacteria to produce antimicrobial agents was tested on standard strains of fungi species of Candida albicans (ATCC® 10231™) and bacterial species of Escherichia coli (ATCC® 8739™), Pseudomonas aeruginosa (ATCC® 9027™), Staphylococcus aureus (ATCC® 6538™), Bacillus subtilis (ATCC® 6633™), Staphylococcus epidermidis (ATCC® 12228™), Klebsiella pneumoniae (ATCC® 4352™), Enterococcus hirae (ATCC® 10541™), vancomycin-resistant Enterococcus faecalis (VRE) (ATCC® 51299™) and methicillin-resistant Staphylococcus aureus (MRSA) (ATCC® 33591™).

Bacterial suspensions containing 3 × 10⁸ cells ml⁻¹ of the selected isolates were prepared. 2.5 µl of each suspension were incubated on Mueller Hinton Agar (MHA) plates at 20°C for 24 h. After incubation, all media in which bacterial colonies were observed, were exposed to ultraviolet (UV) radiation in an
open laminar flow cabinet. Therefore, the vitality of the bacteria was destroyed. 1.5 × 10^8 cells ml^{-1} of 24 h fresh cultures of the standard strains were prepared. 100 μl of each suspension was mixed with TSA medium at 45°C. Subsequently, it was poured into the previously UV exposed plates, then incubated for 24 hours at 37°C after solidification. At the end of the incubation period, the growth of the standard bacteria in the TSA was investigated and the zone diameters were measured (24).

2.7 Susceptibility to Antibiotics

The sensitivity of 101 selected isolates to antibiotics was examined by using the disc diffusion method of Kirby-Bauer (21) in which 10 antibiotics were used: piperacillin (100 μg), erythromycin (15 μg), vancomycin (30 μg), ampicillin (10 μg), neomycin (10 μg), gentamycin (10 μg), chloramphenicol (30 μg), tetracycline (10 μg), rifampicin (30 μg) and ofloxacin (10 μg). The incubation conditions were 24 h at 20°C. *Escherichia coli* (ATCC® 8739™), *Pseudomonas aeruginosa* (ATCC® 9027™) and *Staphylococcus aureus* (ATCC® 6538™) were tested against the same antibiotics as control microorganisms (25).

3. Results and Discussion

3.1 Physicochemical Measurements of Sampling Areas

Temperature, pH, conductivity and hardness values of water samples are shown in Table I. The air temperature of the sample areas A, B, C (Figure 1) was determined. The temperature of area A was 9.8°C and that of B and C were determined as 9.4°C. The moisture value was evaluated as 93% in all these areas. The Parsık cave resembles most cave systems with its high level of humidity and stable air temperature (26, 27). It was determined that the pH and hardness values of the waters at points B and C were higher than those at point A.

These details highlight the differences in chemical environment that may exist within the cave areas.

3.2 Number of Determined Total (Live/Dead) Bacteria

The highest vitality percentage of bacteria isolated in soil samples was found in samples from point B with 38.7%, whereas the highest vitality percentage in the water samples was found in samples from point C with 26.3% (Table II). In cave environments, it is observed that bacteria can survive metabolically but cannot be cultured. This is because bacteria enter a viable but nonculturable cell form under extreme environmental conditions such as low or high temperature, nutrient deficiency, osmolarity and light. In addition, cave microorganisms obtain their energy from the cave atmosphere or the cave surfaces to which they are attached (28, 29).

3.3 Number and Classification of Culturable Aerobic Heterotrophic Bacteria

SCA, ISP4, SEA and AIA-G have been used especially in surface and soil samples to increase the probability of isolating bacteria belonging to phylum Actinobacteria, which have an extremely high potentials in terms of antimicrobial production (30). TWA and R2A medium were used for both isolation and counting of other bacterial groups. Apart from these media, 1/2 TSA was used for isolation of other bacterial groups from all samples. The cave environment in general is oligotrophic and these media provide a similar environment to the culturable cave bacteria. The number of culturable aerobic heterotrophic bacteria from water and soil samples obtained from R2A and TWA media is given in Figure 2.

When the bacterial counts of water and soil samples in R2A and TWA media were examined, the highest bacterial numbers were found in R2A medium. These results were evaluated statistically using the Kruskal-Wallis test. The \( p \) value was found to be 0.09 and no significant difference was found between the numbers of bacteria grown on the R2A and TWA media. In a study conducted in 2014 (31), the efficiency of various media (SEA, TWA, SCA, TSA) was compared to their suitability for bacterial counting. Efficient results for both isolation and counting were obtained in TWA.

A total of 372 bacteria were isolated from all samples. VITEK® analysis was applied to only 321 bacteria which had different characteristics in

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Table I: Physicochemical Measurements of Water Samples

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culture-based analyses. The results of the systematic classification of the bacteria were compiled by biochemical analysis using the VITEK® 2 Compact automated system. Actinobacteria (33%) was determined to be the dominant phylum in this study while the other determined phyla were Firmicutes (25%) and Proteobacteria (16%). In our previous work in Kadıini cave in Turkey, the dominant phylum was Firmicutes (86%), followed by Proteobacteria (12%) and Actinobacteria (2%) respectively (32). In addition, in the study done by Tomova et al. (33), Proteobacteria (51.45%) were found to be the dominant phylum in the samples taken from the Magura cave, Bulgaria, followed by Actinobacteria (43.68%) and Bacteroidetes (3.88%). Although the bacterial habitat of each cave is specific, Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes are the most identified groups in culture-based microbiological studies in caves (34–36).

In our study, Firmicutes was the most common phylum in soil samples with a rate of 33%, while the most common phylum determined in surface and water isolates was Actinobacteria with 36% and 35% respectively. Considering all the samples, at the class level, Actinobacteria was the most dominant with 33%, while Bacilli with 23% was detected as the second dominant class. It was demonstrated through previous studies that Actinobacteria existed mainly in cave walls, soil, sediment and on speleothem surfaces, which might have considerably contributed to the formation of cave structures and the biomineralisation in the cave ecosystems (4–37). Actinobacteria as well as Firmicutes are frequent among the microbial population inhabiting the caves. Compared to the Proteobacteria group, Firmicutes are more resistant to stress caused by dehydration as well as restriction of nutrients (37). Contrary to our findings for Parsık cave, Proteobacteria are a dominant group in heterotrophic bacterial communities in many caves (33, 34, 38–40). In the current study, Proteobacteria were determined respectively as 10%, 21% and 17% in the surface, water and soil samples. The dominant classes of this phylum were found to be Gammaproteobacteria and Alphaproteobacteria with 9.2% and 6.4%, respectively. In our previous study in Kadıini cave, Alphaproteobacteria were detected at 2%, while Gammaproteobacteria were at 9% (32). The phylum Proteobacteria, having a key role in biogeochemical cycles, and being abundant in samples from cave sediment, soil, dripping water and cave surface, is a cosmopolitan bacterial group (37).

### 3.4 Enzymatic Reactions of Parsık Cave Bacteria

Enzymatic reactions of microorganisms give us ideas of their metabolic activities which are related to their environment. The biochemical tests of our isolates in the VITEK® system were not only useful for bacterial identification but...
also to provide more information about nutrients in Parsık cave. In addition, results of these tests were used to evaluate the potentials of the isolates for biotechnological uses in terms of their enzyme production. 76 Gram-negative bacteria, 194 Gram-positive and 51 Gram-positive spore forming bacteria have been tested using the GN, GP and BCL cards respectively in the VITEK® 2 compact device, and results are given in Figure 3, Figure 4 and Figure 5 respectively. Most of the isolates displayed peptidase (arylamidase) while only Gram-negative bacteria (less than 10%) showed lipolytic activity. In the study conducted in Gumki cave, India, 75.5% of bacteria produced lipase, 47% were amylase producers and 24% produced protease (41). Another study screening cave bacteria for enzyme production found 40% lipase and 87% protease producers (33). This variation in enzymatic profiles in cave bacteria reinforces the idea that every cave is unique.

The high activity of amino acids arylamidase determined in our tested isolates indicates their potential for protein catabolism (42). The phyla Firmicutes (31%) and Actinobacteria (30.7%) produced the highest amounts of arylamidases identified among the tested isolates. 85.52%, 65.97% and 82.35% of Gram-negative, Gram-positive and Gram-positive spore forming bacilli revealed tyrosine-arylamidase activity. Tyrosine is a non-essential amino acid which is synthesised through phenylalanine hydrolysis. It plays a major role in most enzyme synthesis as reported by Kalkan and Altuğ (42), since it is the phosphate and sulfate receptor of protein kinase during protein synthesis. It is also used to reinforce the activity of proteins as demonstrated in a study conducted in thrombin inhibitors showing that tyrosine sulfation could open a way for the development of an anti-thrombotic drug (43). Hence, tyrosine arylamidase has a valuable role in biotechnology since it contributes to the liberation of the amino acid tyrosine.

Enzymes like leucine arylamidase have been reported to be important in food processing industries and the treatment of waste products (44, 45). The degradation of leucine and other amino acids results in volatile molecules responsible for the flavours of some foods like meat products as reported by Papamanoli et al. (46) and Lee et al. (44). In addition, a study showed the roles of bacteria in conversion of paper mill sludge demonstrating the important contribution of amino acid peptidase with leucine arylamidase (45). In our study, 81.95% and 88.23% of Gram-positive bacilli
bacteria and Gram-positive bacilli showed leucine arylamidase activity. This enzyme was the second most produced enzyme, after the tyrosine arylamidase, by our isolates. Bacteria which can produce this enzyme could be used directly or indirectly by using their enzymes in both composting of sludge and fermentation of food products such as meat and dairy products.
VITEK® results have showed that some Parsik cave isolates exhibit beta-galactosidase activity which is the more expressed carbohydrate hydrolase in this study. Considering the whole of the tested isolates, most of the bacteria producing beta-galactosidase belong to the Firmicutes phylum with 40.6%, while only 10.9% of beta-galactosidase producers were classified under the phylum Proteobacteria.

The main role of the beta-galactosidase enzyme is to convert lactose into monosaccharides. Glucose and galactose resulting from this reaction not only contribute to the development of the cell but can also be used in dairy product processing (44, 47). This enzyme is important since it solves the problem of human lactose intolerance. The hydrolysis of lactose by this enzyme results in molecules like galactooligosaccharides which have health benefits as prebiotics (47). Moreover, breakdown of some sugars like D-mannose, D-mannitol and D-glucose by fermentation was reported, especially in Gram-negative bacteria.

Lipolytic activity was also observed in some of our isolated Gram-negative bacteria (less than 10%). Even if it was produced by a minimum number of isolates, the activity of lipase was fully expressed by bacteria belonging to the phylum Proteobacteria. This class of enzymes which is used in hydrolysis of lipids could be important in bioremediation since it could participate in oil degradation. Sharma et al. reported that microbial lipases are best for biodiesel production (48). Since they can use all types of free fatty acids and glycerides, they exhibit a high activity, thermostability, alcohol resistance, less reaction time as well as less production inhibition (48). Other enzymes were produced by some of the bacteria in Parsik caves. Further studies should be carried out to clarify them and assess their biotechnological uses.

### 3.5 Antimicrobial Agent Production Capability

Microorganisms with broad-spectrum bioactive components, antifungal and antibacterial agents in cave-specific habitats are common in these extreme environments (17). In our study, a total of 129 cave bacteria were tested for their antimicrobial effect against nine different standard bacterial strains and one fungal strain. Experiments have shown that 10 of the selected bacteria (six from Actinobacteria class, four from Bacilli class) have antimicrobial effects against the standard strains. Parsik cave isolates displayed variable inhibition rates against the tested microorganisms and the highest inhibition rate was observed against Candida albicans. Some of our cave isolates have been found to have inhibitory effects against S. aureus, S. epidermidis, VRE and P. aeruginosa. The zone diameters of cave bacteria with antimicrobial properties against tested microorganisms are shown in Table III.

In our study, the isolate which affected S. epidermidis belongs to the Bacilli class and those which inhibit VRE and S. aureus belong to the Actinobacteria class. Some studies have shown that bacteria with antimicrobial activity inhabiting karst caves are often from the Actinobacteria class (30, 31). However, cave bacteria belonging to the firmula Proteobacteria, Firmicutes (especially Bacilli class) and Bacteroides were determined to have antimicrobial and bioactive substances. Thus, approximately 50% of the bacteria isolated from the Magura cave, Bulgaria were detected to inhibit the increase of P. aeruginosa (33). Cave bacteria inhibiting MRSA and VRE clinical strains were determined in a study on Actinomycetes isolated from 19 different caves in Turkey (30). Certainly the bacteria belonging to the class Actinobacteria are the best known in terms of antimicrobial material synthesis, but the isolation of bacteria belonging to the other classes is very important especially in karst environments.

### 3.6 Determination of Antibiotic Resistance Profiles of Isolated Bacteria

Antibiotic resistant bacteria are widespread in several environments. In this study, resistance to 10 different antibiotics of 101 bacteria (76% Gram-positive; 25% Gram-negative) selected from the cave isolates was investigated. Isolates with a metabolic reaction rate of at least 95% similarities to the data in the VITEK® database were selected. When the antibiotic resistance profiles of the isolates were examined, 7% of the bacteria belonging to the cave were resistant to all antibiotics. The highest number of bacteria showed resistance against ampicillin with a rate of 38.6%. In addition, 35.6% of the isolates showed resistance against two or more antibiotics.

Antibiotic resistance profiles of Gram-positive and Gram-negative cave isolates are shown in Figure 6. The lowest resistance was observed to rifampicin (9% for Gram-positive and 8% for Gram-negative). In parallel with our study, it was determined that all the Pajsarjeva jama, Slovenia, isolates were sensitive to rifampicin (49). Likewise, low levels
### Table III Antimicrobial Agent Production Ability

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ND = not determined
of resistance to ofloxacin, which is a DNA/RNA synthesis inhibitor like rifampicin, were observed in Parsik cave isolates (11% in Gram-positive and 12% in Gram-negative). The resistance rate of Pajsarjeva jama isolates to erythromycin was 73.6% for Gram-negative and 39% for Gram-positive bacteria. The resistance of Parsik cave isolates to erythromycin was determined at lower levels of 20% and 21% for Gram-negative and Gram-positive bacteria respectively. The levels of resistance to protein synthesis inhibitors other than erythromycin (gentamycin, neomycin, tetracycline and chloramphenicol) were determined to range from 12% to 20% for both Gram-positive and Gram-negative bacteria. Contrary to our study, Lavoie et al. (50) showed that cave isolates were highly resistant to gentamycin, neomycin and chloramphenicol antibiotics (33–66% for Gram-negative bacteria and 61–83% for Gram-positive bacteria).

Furthermore, the lowest resistance to cell wall synthesis inhibitors was observed in piperacillin for both Gram-positive (12%) and Gram-negative (16%) bacteria. In the study conducted by Lavoie et al. (50), the resistance to piperacillin, compared to other antibiotic resistance, was found to be lower (average 37.5%).

Fig. 6. Resistance levels of Parsik cave bacteria against various antibiotics which are grouped according to their mode of action: (a) Gram-positive isolates; (b) Gram-negative isolates
In our study, considering the cell wall synthesis inhibitors (vancomycin and ampicillin), Gram-negative bacteria were found to be more resistant than the Gram-positive ones. Similar to our study, Avguštin et al. (49) revealed that cave Gram-negative isolates showed higher resistance to ampicillin.

According to VITEK® results, except ampicillin and vancomycin, Actinobacteria were determined to be the most resistant (47–70%) phylum to all tested antibiotics. The highest resistance to ampicillin and vancomycin was observed in the phylum Proteobacteria. Like the microbial diversity of caves, antibiotic resistance is also variable. While the antibiotic resistance rates were high, no isolate producing antimicrobial agent was detected in the study conducted by Lavoie et al. (50). However, one of the antibiotic resistance hypotheses in caves is that there is a high rate of antibiotic resistance in the presence of microorganisms producing antimicrobial agents. Studies have shown that bacteria having antibiotic genes can also produce antimicrobial agents (51, 52). In our study, it was found that 50% of the isolates producing antimicrobial agents were resistant to at least two antibiotics. Therefore, study of bacterial antibiotic resistance may contribute to the development of new antibiotics. To clarify this issue, studies in this issue should be continued.

4. Further Work

In our future studies, we are planning to purify and use the enzymes and antimicrobial substances produced by these isolates. Apart from the potential of bacteria isolated from Parsık cave to produce enzymes and antimicrobial agents, it is planned to determine their potential use in biodegradation, self-healing concrete and production of antimicrobial drugs against phytopathogens and entomopathogens.

5. Conclusion

The microorganisms attached to the specific environmental conditions of caves are important in terms of exploring their uses and specific features. This study was the first microbiological study in Parsık cave. It has been demonstrated that enzymes such as aroylamidases, carbohydrate hydrolyses and lipases found in bacteria isolated from Parsık cave can be important in industrial as well as clinical fields. In addition, some of our isolates have shown antimicrobial activity and can contribute to the development of new antibiotics. Antibiotic resistance profiles of Parsık cave isolates should be clarified in further studies through studies of their genes.

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Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>5KG</td>
<td>5-keto-D-gluconate</td>
</tr>
<tr>
<td>ADH1</td>
<td>arginine dihydrolase 1</td>
</tr>
<tr>
<td>ADH2s</td>
<td>arginine dihydrolase 2</td>
</tr>
<tr>
<td>ADO</td>
<td>Adonitol</td>
</tr>
<tr>
<td>AGAL</td>
<td>alpha-galactosidase</td>
</tr>
<tr>
<td>AGLTp</td>
<td>glutamyl aroylamidase pNA</td>
</tr>
<tr>
<td>AGLU</td>
<td>alpha-glucosidase</td>
</tr>
<tr>
<td>AIA-G</td>
<td>Actinomycetes isolation agar</td>
</tr>
<tr>
<td>AlaA</td>
<td>alanine aroylamidase</td>
</tr>
<tr>
<td>AMAN</td>
<td>alpha-mannosidase</td>
</tr>
<tr>
<td>AMY</td>
<td>D-amygdalin</td>
</tr>
<tr>
<td>APPA</td>
<td>Ala-Phe-Pro-arylamidase</td>
</tr>
<tr>
<td>AspA</td>
<td>L-aspartate aroylamidase</td>
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<tr>
<td>BACI</td>
<td>bacitracin resistance</td>
</tr>
<tr>
<td>BAAlap</td>
<td>beta-alanine aroylamidase pNA</td>
</tr>
<tr>
<td>BCL</td>
<td>Gram-positive spore-forming bacilli</td>
</tr>
<tr>
<td>BGAL</td>
<td>beta-galactosidase</td>
</tr>
<tr>
<td>BGAR</td>
<td>beta-galactopyranosidase</td>
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<tr>
<td>BGLU</td>
<td>beta-glucosidase</td>
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<tr>
<td>BGUR/</td>
<td>BGURr</td>
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<tr>
<td>BNAG</td>
<td>beta-N-acetyl-glucosaminidase</td>
</tr>
<tr>
<td>BXYL</td>
<td>beta-xilosidase</td>
</tr>
<tr>
<td>CDEX</td>
<td>cyclodextrin</td>
</tr>
<tr>
<td>CIT</td>
<td>citrate (sodium)</td>
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</table>
CMT  coumarate
CTC  5-cyano-2,3-ditolyl-tetrazolium chloride
DAPI  4',6-diamidino-2-phenylindole
dCEL  D-cellobiose
dGAL  D-galactose
dGLU  D-glucose
dMAL  D-maltose
dMAN  D-mannitol
dMLZ  D-melezitose
dMNE  D-mannose
dRAF  D-raffinose
dRIB  D-ribose
dSOR  D-sorbitol
dTAG  D-tagatose
dTRE  D-trehalose
dXYL  D-xylose
ELLM  Ellman
ESC  esculin hydrolysis
GGAA  Glu-Gly-Arg-arylamidase
GGT  gamma-glutamyl-transferase
GlyA  glycine arylamidase
GLYG  glycogen
GN  Gram-negative fermenting and non-fermenting bacilli
GP  Gram-positive cocci and non-spore-forming bacilli
GPS  Global Positioning System
IARL  L-aribitol
IHISa  L-histidine assimilation
ILATk  L-lactate alkalinisation
IMLTa  L-malate assimilation
INO  myo-inositol
INU  inulin
IRHA  L-rhamnose
ISP4  inorganic salt-starch agar
KAN  kanamycin resistance
LAC  lactose
LDC  lysine decarboxylase
LeuA  leucine arylamidase
LIP  lipase
LysA  L-lysine-arylamidase
MBdG  methyl-beta-D-glucopyranoside
MHA  Mueller Hinton Agar
MNT  malonate
MRSA  methicillin-resistant *Staphylococcus aureus*
MTE  maltotriose
NAG  N-acetyl-D-glucosamine
NAGA  beta-N-acetyl-galactosaminidase
NC6.5  growth in 6.5% NaCl
NOVO  novobiocin resistance
O129R  O/129 resistance (comp. vibrio.)
ODC  ornithine decarboxylase
OFF  fermentation/glucose
OLD  oleandomycin resistance
OPTO  optochin resistance
PHC  phosphoryl choline
PheA  phenylalanine arylamidase
PHOS  phosphatase
PIPLC  phosphatidylinositol phospholipase C
PLE  Palatinose™
POLYB_R  polymyxin B resistance
ProA  L-proline arylamidase
PUL  pullulan
PVATE  pyruvate
PyrA  L-pyrrolidonyl-arylamidase
R2A  Reasoner’s 2A agar
SAC  saccharose/sucrose
SAL  salicin
SCA  starch casein agar
SEA  soil extract agar
SUCT  succinate alkalinisation
TSA  1/2 tryptic soy agar
TTZ  tetrazolium red
TWA  tap water agar
TyrA  tyrosine arylamidase
URE  urease
UV  ultraviolet
VRE  vancomycin-resistant *Enterococcus faecalis*

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