İSTANBUL UNIVERSITY PRESS

European Journal of Biology
Eur J Biol 2023; 82 (2): 224–238
DOI: 10.26650/EurJBiol.2023.1247822

REVIEW ARTICLE

Pseudomonas otitidis: Discovery, Mechanisms and Potential Biotechnological Applications

Gao Jianfeng[1,2], Rosfarizan Mohamad[1,2], Murni Halim[1,2], Mohd Shamzi Mohamed[1,2]

[1]Universitiy of Putra Malaysia, Department of Bioprocess Technology, Faculty of Biotechnology & Biomolecular Sciences, Selangor, Malaysia
[2]Universitiy of Putra Malaysia, Bioprocessing and Biomanufacturing Research Complex, Faculty of Biotechnology & Biomolecular Sciences, Selangor, Malaysia

ABSTRACT

Pseudomonas otitidis is a species of *Pseudomonas* bacteria discovered in the early 2000s and has been studied systematically by many researchers. *P. otitidis* has been isolated from various infected parts of diseases, such as otitis, recurrent pneumonia, necrotizing fasciitis, peritonitis, foot cleft, or burns. It has been found to produce a variety of enzymes to decompose pollutants in the environment such as petroleum, polycyclic aromatic hydrocarbons, dyes, sodium dodecyl sulfate, zearalenone, etc. Furthermore, it can produce some ingredients for application in agriculture and health industries such as digestive enzymes, melanin, and L-asparaginase. Some scholars used *P. otitidis* as a model organism to investigate environmental degradation, biobattery, plant growth promotion, and biodegradable plastic polyhydroxyalkanoate production. The biofilm of *P. otitidis* consists of rhamnolipid. The research has provided the basis to produce rhamnolipid and the effective removal methods of *P. otitidis*. *P. otitidis* is prone to resistance to lactam antibiotics, and its resistance is caused by its unique metallo-β-lactamase, a polyoxometalate enzyme. In other words, *P. otitidis* is a very interesting bacterium candidate to be used in different research fields. Hence, in this paper, the discovery, mechanisms, and potential biotechnological applications of *P. otitidis* are described.

Keywords: *Pseudomonas* sp., *Pseudomonas otitidis*, Metallo-β-lactamase, Biofilm, L-asparaginase, Biosurfactant

INTRODUCTION

Pseudomonas sp. is a type of Gram-negative strain, which don't possess a cell nucleus and is unable to form spores. It is generally rod-shaped, with one to more flagella. It is easy to culture in vitro conditions, and it can secrete a variety of pigments. Most of the pigments are characteristic of *Pseudomonas* sp. and have antimicrobial properties. *Pseudomonass* sp. is a heterotroph bacterium that can utilize organics and an aerobic bacterium, which metabolizes through respiration. Although it is an obligate aerobe, it can grow anaerobically during nitrate reduction. *Pseudomonas* sp. is present and spread widely in the environment.*Pseudomonas* sp. genus was described as a whole group consisting of more than 100 known species.[1] The rRNA-DNA hybridization method was used to categorize this genus into five groups.[2] Kersters gave a new definition according to its previous classification studies.[3] In the past decades, genetic and molecular techniques played an important role in the classification of Pseudomonas [4] and many species were reclassified again.[5]

The diversity of habitat range, biological shape, and versatile function attracted microbiologists to study it for a long time.[6] Several *Pseudomonas* species can cause disease in humans and animals, like pulmonary infection,[7] respiratory tract infection,[8] bacteremia,[9] and urinary infection.[10] Serious medicine resistance problem exists on *Pseudomonas sp.* The medicine resistance of *Pseudomonas* is related to its ability to produce some enzymes that degrade antibiotics. The enzymes and the relevant genes have been widely studied such as the enzymes *Pseudomonas otitidis* metallo-β-lactamase (POM),[11] Pseudomonas fluorescens metallo-β-lactamase (PFM),[12] or São Paulo metallo-β-lactamase (SPM)[13] that mediate the efficient hydrolysis of carbapenems. Metallo-β-lactamase (MBL) is a kind of enzyme that can cause hydrolysis of carbapenem. It includes the POM enzyme produced by *Pseudomonas otitidis*, the (PAM) enzyme produced by *P. aeruginosa*, L1 enzyme of *Stenotrophomonas maltophilia* et al. Differences in their nucleotide sequences lead to differences in drug resistance and leading to evolutionary diversity.[14] Many functional genes of the *Pseudomonas* genus are similar, it is probably related to the phages that infect them. For example, the pf20 phage derived from Olsen's PX4 is capable of inter-infecting *P. aeruginosa* and *P. putida*.[15] Some phages remain as plasmid-like phages after entering *Pseudomonas*, such as pf16H2 in *P. putida*.[16] It leads to the high similarity between many gene sequences of *Pseudomonas sp*, and the evolution of *Pseudomonas*

Corresponding Author: Rosfarizan Mohamad **E-mail:** farizan@upm.edu.my
Submitted: 21.03.2023 • **Revision Requested:** 20.05.2023 • **Last Revision Received:** 19.09.2023 • **Accepted:** 06.10.2023 • **Published Online:** 07.12.2023

sp. Identification of *Pseudomonas* bacteria is usually achieved by 16srDNA sequencing.[17] However, this method can occasionally lead to high similarity between a newly isolated strain and other existing *Pseudomonas* strains. So, it is difficult to determine the attribution of the new strain.[18] For example, *P. aeruginosa* has a strong similarity with *P. otitidis* MrB4, the similarity between them determined by the 16srDNA sequence is 98.6%, but they are in different taxonomic positions.[19] Some studies have analyzed the gyr gene sequence to determine the attribution of new strains.[20] Manivannan et al. isolated a strain and mapped its genome. They proposed that *P. aeruginosa* is a hypothesized species based on sequence homology, which is closest to the homology of *P. otitidis*.[21] In addition to 16SrRNA testing, MALDI-TOF MS Biotyper methods were used by Kačániová et al. for *Pseudomonas*' genotypic identification. It revealed a high discriminatory power of the MALDI-TOF MS Biotyper methods for the identification of *Pseudomonas* sp.[22]

Pseudomonas otitidis

P. otitidis is a novel species of *Pseudomonas sp*, which was discovered in 2002. In clinical studies conducted between 1998 and 2000, 101 researchers in the United States collected microbial samples from 2,039 patients (2,240 diseased ears). A total of 2838 bacteria strains were detected from 2048 ears and clinically diagnosed as acute otitis. Researchers isolated a new strain of *Pseudomonas* genus, closely related to *P. aeruginosa*. It was named *P. otitidis* by Roland et al.[23] In the following years, there were few studies on *P. otitidis* until 2006, when Clark et al. isolated 41 new strains of *P. otitidis* from the affected parts of patients with acute otitis.[24] Using DNA-DNA hybridization, 16S rRNA sequencing, and phenotypic analysis, researchers found that these *Pseudomonas* did not match any other known *Pseudomonas* species except *P. otitidis*. Researchers carried out systematic research about morphological tests, physiological and biochemical tests, drug sensitivity tests, fatty acid identification, and DNA component proportion determination of isolated *P. otitidis*.[24] Due to the systematic study of Clark et al., many researchers also considered that Clark et al. first discovered *P. otitidis* in 2006.[19,25] Clark et al. were the first to study the biochemical and physiological properties of ten *P. otitidis* strains as described below; Nine of ten strains can produce pyocyanin, nine of ten strains can produce fluoroscein, none of the strains can be cultivated at 4 ℃ or 47 ℃, five of ten strains can growth at 7 ℃. None of the strains can produce urease, eight of ten strains can hydrolyze gelatin and no strain can hydrolyze casein. Nine strains can growth on 4% sodium chloride agar, eight stains can grow on 5% sodium chloride agar. Ten strains all can't utilize N-Acetyl-D-glucosamine, D-Arabitol, Glycerol, D-Mannitol, D-Sorbitol, D-Fructose, L-Fucose, D-Galactose, Gentiobiose, Maltose, Sucrose, D-Trehalose, D-Xylose, D-Galacturonic acid, D-Gluconic acid, D-Glucuronic acid, Itaconic acid, Itaconic acid, L-Phenylalanine, D-Serine, Cytosine, Acetic acid, Sebacic acid, Inosine and Uridine. They can utilize L-Arginine, L-Histidine, L-Isoleucine, L-Leucine (9/10), L-Ornithine, L-Serine, β-Phenylethylamine, γ-Aminobutyric acid, Phenylethylamine and Putrescine.

Many scholars and doctors have isolated *P. otitidis* strains from infected people. Kim et al. reported two cases of infection in 2016. One is necrotizing fasciitis complications, another is peritonitis.[25] In 2015, researchers isolated 10 strains from the specimens of foot cleft patients, which were identified as *P. otitidis*.[26] In 2020, Japanese researchers sequenced the whole genome of *P. otitidis* TUM18999 from burn patients. The TUM18999 was identified as *P. otitidis*. Other researchers detected a novel specific B3 sub-group BML from this strain. Its similarities with *P. alcaligenes*' PAM-1 and *P. otitidis* TUM18999's POM-1 were 90.24% and 73.14%, respectively.[27] In 2021, Denmark physicians reported a case of known moderate chronic obstructive pulmonary disease with bronchiectasis and recurrent pneumonia. Blood cultures showed growth of *P. otitidis*. Researchers noted in the study that the infection caused by this new pathogen easily causes misdiagnosis.[28] In addition to being isolated from patients, *P. otitidis* also exists widely in nature. At present, some researchers have studied *P. otitidis* isolated from nature. Miyazaki et al. isolated a *P. otitidis* MrB4 strain from Lake Biwa, Japan, and sequenced its whole genome.[19]

P. otitidis can utilize and decompose many pollutants in the natural environment, such as crude oil, polycyclic aromatic hydrocarbons, dyes, sodium dodecyl sulfate (SDS), zearalenone, etc. It also can produce some useful substances, such as digestive enzymes, melanin, and L-asparaginase. Some scholars studied *P. otitidis*' biofilm, including the mechanism of biofilm production and the comparison between *P. otitidis* biofilm and other *Pseudomonas* biofilm. Some researchers used the bacterium to promote plant growth, research on biological batteries, and the production of polyhydroxyalkanoates (PHA). Metallo-β-lactamase produced by *P. otitidis* does not show intrinsic resistance to the carbapenem antibiotics. Figure 1 shows the major research areas of *P. otitidis*. The diagram depicts eight hot areas of research on *P. otitidis* and some achievements have been made in these fields.

Stability of *Pseudomonas otitidis* in Ecosystem

Some researchers are interested in the stability of *P. otitidis* in the ecosystem. García-Ulloa et al. studied *P. otitidis* in Cuatro Cienegas overexploitation of the agricultural wetland ecosystem in Coahuila, Mexico. As the water fades due to overexploitation. *P. otitidis* in the water loses metabolic complexity and diversity. In the following year, *P. otitidis* became extinct in this wetland ecosystem.[29] In addition, the researchers also analyzed the bacterial population history and evolutionary response of bacterial lineages to disturbances by comparing the

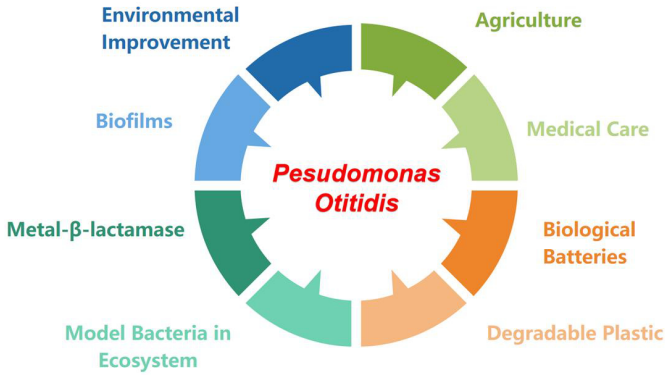

Figure 1. Research areas of *P. otitidis*.

genomes of a population sample.[30] Another study speculated on the eventual decline or possible extinction of *P. otitidis*. They propose that phosphorylation, DNA recombination, changes in small molecule metabolism and transport due to environmental disturbances, and loss of biosynthetic and regulatory genes lead to changes in bacterial populations.[31] Rodríguez-Verdugo et al. investigated the seasonal diversity of culturable *Pseudomonas* bacteria in lagoons of the Chihuahuan Desert, Mexico. They isolated 70 *Pseudomonas* strains collected from lagoons on four independent days. *P. otitidis* and *P. cuatrocienegasensis* accounted for 64% of the total number of isolates. More interestingly, *P. otitidis* was isolated only in summer, while *P. cuatrocienegasensis* was isolated only in winter due to the different growth conditions of *Pseudomonas* sp.[32]

Jun et al. isolated 21 populus-associated *Pseudomonas* isolates from the roots of poplar trees and through genetic analysis, it can be divided into three subgroups. The gene specificity of subgroup 1 includes several sensory systems that play a role in two-component signal transduction and depend on receptors. The gene specificity of the second subgroup included putative genes, and the specific genes of the third group were annotated as having hydrolase activity. The only isolated *P. otitidis* strain was different from the three subgroups.[33] Dahiya and Mohan thought that in the same ecological environment system, different microorganisms could improve and degrade different pollutants, and the addition of external microorganisms with a high degradation rate could significantly improve the process efficiency. In their study, *P. otitidis, Bacillus firmus, Bacillus subtilis* and *Bacillus circulans* were isolated and used to improve chemical oxygen demand (COD) and degrade nitrate, phosphorus, and moldy volatile fatty acids.[34]

Degradation of Organics

The degradation of harmful organics in wastewater and soil is a research focus of *P. otitidis*, such as the degradation of some components of oil pollution. Dasgupta et al. isolated a crude oil-degrading bacteria *P. otitidis*. In their research, the biodegradability of crude oil was evaluated by gas chromatography, and the formation of biofilm near the oil-water interface was quantitatively analyzed by a confocal laser scanning microscope. *Pseudomonas* supported by biofilms was found to degrade crude oil more easily and extensively than plankton. Moreover, *P. otitidis* biofilms formed in the presence of crude oil accumulated higher biomass and thicker biofilms.[35] Gogoi et al. isolated a strain of *P. otitidis* DU-13 capable of degrading oil pollution and water restoration and found that after 7 days of culture and treatment, the biosurfactant produced by the isolated strain could reduce the surface tension of oil-containing medium by 46%.[36] Peng et al. isolated a *P. otitidis* strain (Strain 81F, Accession Number AB698739.1). The degradation activity of polyurethane (PU) was evaluated. Their study describes the activity of *Pseudomonas* to degrade PU at a high level and describes the enzymes involved in this process, a 45 kDa product with PU enzyme activity. It plays an important role in bioremediation and plastic waste treatment.[37] Bestawy et al. investigated the treatment of industrial wastewater by the combination of bacterial consortium and Fe_3O_4 magnetic nanoparticles. Glycine-coated magnetic nanoparticles (Fe_3O_4 NPs) were prepared by reverse coprecipitation and characterized by X-ray diffraction, transmission electron microscopy, scanning electron microscopy, and vibrating sample magnetometer. Exogenous mixers include *Enterobacter cloacae* and *P. otitidis*. The mixture proved to have a good improvement effect on COD, oil, and grease (O&G), and total petroleum hydrocarbons.[38]

Removal of phenolic compounds from waste alkali by *P. otitidis* has been studied.[39] Waste alkali is one of the phenolic industrial pollutants produced by chemical processes in petrochemical plants and refineries. It has high COD, and pH, and contains high salinity and sulfides. The *P. aeruginosa* strain that can degrade phenol was isolated in previous studies. Mohammadi and collegous' study is the first to isolate *P. otitidis* capable of decomposing phenol.[39] Maitra et al isolated and identified phenol-degrading bacteria from soil, identified as *P. otitidis*.[40] Mohanty and Jena isolated a strain (*Pseudomonas* sp. Strain NBM11) from soil samples contaminated with medical waste and wastewater. The degradation capacity of the strain to phenol was up to 1000 mg/L by optimizing the culture conditions.[41]

Poyraz isolated a *P. otitidis* strain capable of degrading toluene from a sewage treatment plant, and researchers measured their tolerance and biotechnological potential.[42] Dados et al. proposed rapid remediation methods for soils heavily contaminated by organics. One of the methods uses contaminated soil as a culture material to enrich strains that degrade soil organic matter and then use those strains for soil remediation. Two *Pseudomonas* strains were isolated, EL20 and EL15 respectively, one of them was *P. otitidis*, which could degrade total petroleum hydrocarbons and N-alkanes *in vitro*.[43] Anwar et al. isolated several novel strains, including *P. otitidis*. The degradation ability of polyvinyl chloride under different cultures and time intervals was studied using scanning elec-

tron microscopy, atomic force microscopy, UV-visible spectroscopy, Fourier transform infrared (FT-IR), gel permeation chromatography, and differential scanning calorimetry.[44] Xu et al. isolated a *P. otitidis* strain W12 using phenanthrene as a carbon source from the activated sludge in the aeration tank of the north Shenyang sewage treatment plant.[45]

Rhamnolipid Content

Rhamnolipid is one of the most characteristic biosurfactants.[46] It is a surface-active molecule produced by *Pseudomonas* using various organic compounds.[47] Biosurfactants are divided into 4 types, they are cationic type, anionic type, zwitterionic type, and non-ionic type separately. Rhamnolipid is an anionic biosurfactant. Its hydrophilic end is composed of 1-2 molecules of rhamnose, and the hydrophobic end is composed of lipid structures.

Rhamnolipid is an important component of *P. otitidis* biofilm formation.[48] Haloi et al. isolated a rhamnolipid-produced *P. otitidis* TMB2 strain and described the process of production of rhamnolipid. They characterized the extracted biosurfactants by Fourier Transformed Infrared Spectroscopy and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. They used liquid chromatography-mass spectrometry to detect homologs of single and double rhamnolipids. The thermal stability and degradation modes of candidate biosurfactants were tested by thermogravimetry and differential scanning calorimetry to determine their adaptability.[49]

Buonocore et al. reported for the first time the production of RLs using anthracene and benzene as the only carbon sources using 12 different materials such as monosaccharides, polysaccharides, and petroleum industry derivatives as raw materials.[50] Singh and Tiwary isolated a strain of *P. otitidis* P4 from Chirimi Coal Mine in India and determined the structural properties of BS and the glycolipid properties of BS by biochemical tests, thin layer chromatography, FT-IR and NMR analysis.[51] Before characterizing biosurfactants by FT-IR and NMR, they were purified by column chromatography and confirmed by thin-layer chromatography analysis. Furthermore, scanning electron microscope analysis is also used in morphological characterization.

Degradation of Sodium Dodecyl Sulphate

Ibrahim and Abd Elsalam isolated four strains capable of degrading SDS from Taif wastewater in Saudi Arabia. One strain was identified as *P. otitidis*.[52] Chaturvedi and Kumar isolated 24 SDS-degrading strains from Varanasi, India. The isolated NN1 showed 97% homology with the *P. otitidis* strain RW1, which showed 98% homology with *P. otitidis* TNAU45. The degradation rate at 12h was 19.6% to 97.2%.[53] Jovčlć et al isolated a *P. otitidis* strain (*Pseudomonas sp*. ATCC19151). It was found that it contains a gene encoding sdsA, which is involved in the degradation of SDS.[54] Ibrahim et al. isolated *P. otitidis* and *P. aeruginosa* that could degrade SDS and tried to mutate the strains by UV, ethidium bromide, and biological fixation methods. The degradation efficiency of SDS with alginate immobilized strains was higher than free microbial strains.[55]

Degradation of Organic Dyes

Organic dyes are the main pollution component in urban wastewater and industrial wastewater. Ingestion or exposure to dyes can irritate the respiratory, digestive, and nervous systems, leading to acute and chronic poisoning or disease, and in severe cases cancer. The microbial degradation method of dyes is a research focus at present. Many researchers have studied the ability of some strains of *P. otitidis* to remove stains. Wu Jing et al. used *P. otitidis* WL-13 isolated from the sludge of printing and dyeing wastewater treatment plants to degrade triphenylmethane dye in 2009. When the dye concentration is 500 μmol/L, the removal rate of malachite green and brilliant green can reach 95% when shaking cultured for 12 h, the crystal violet degraded 13% under the same conditions.[56] Jing et al. isolated a new dye decolorization strain, which was identified as *P. otitidis* and named CV-1. It can remove various triphenylmethane dyes and methyl red mono azo dyes.[57] Shah et al. isolated a strain with the ability to decolorize triphenylmethane. The decolorization rate was 72%-96% within 24 h. The strain was *P. aeruginosa* ETL-1, but the similarity between the strain and *P. otitidis* 81F reached 98%.[58] Figure 2 shows several common organic dye pollutants.[59–63]

Metal Settlement and Bioelectrochemistry

Bioelectrochemical system is a promising technology for the removal and recovery of metal ions from acid mine drainage wastewater.[64] The discharge of metal ions in water will lead to environmental pollution and human diseases, especially happen of cancer.[65] Martha et al. isolated mercury-resistant bacteria, including *P. otitidis* from Egyptian wastewater, whose mechanism of action may be related to the *merA* gene. It can reduce the soluble mercury ion to the precipitated monomer mercury. All tested strains contained plasmids encoding the *merA* gene.[66] Furthermore, Yeoh found that *P. otitidis* B1 was able to synthesize siderophores, which isolated iron from the environment.[67] Moreover, Ai et al. isolated *P. otitidis* E8 from a microbial fuel cell anode electroactive biofilm. It can effectively recover copper and cadmium ions from acid mine drainage wastewater when inoculated in a bioelectrochemical system.[68] Besides, Modestra and Mohan found that due to the difference in physical and chemical structure of the cell wall, the electron transfer characteristics of Gram-positive and Gram-negative bacteria may also vary.[69] In addition, Thulasinathan et al. isolated *Cronobacter sakazakii* and *P. otitidis* AATB4 from sewage and conducted a comparative study on the two bacteria

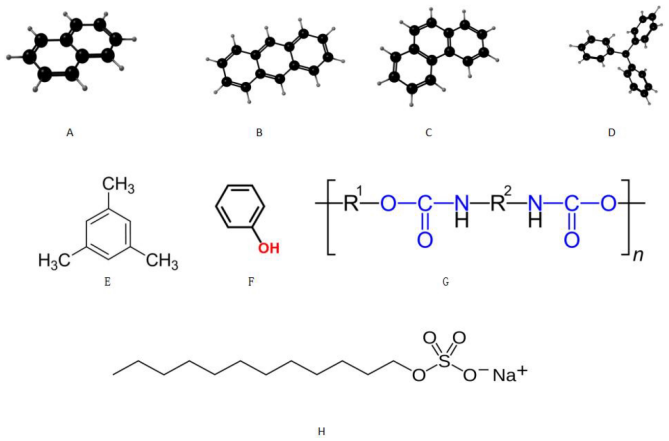

Figure 2. Organic dye pollutants that can be removed by *P. otitidis*. A is naphthaline, B is anthracene, and C is phenanthrene. Phenanthrene is a tricyclic aromatic hydrocarbon with a "bay" region and a "K" region. These two structures are closely related to the carcinogenicity of polycyclic aromatic hydrocarbons. A, B, and C belong to polycyclic aromatic hydrocarbons. Polycyclic aromatic hydrocarbons have toxic, mutagenic, and carcinogenic effects, as well as resistance to biological degradation. Oil spills and environmental pollution impact the food chain in the form of toxic organic materials such as polycyclic aromatic hydrocarbons, posing a major threat to ecosystems including marine life and human beings. Polycyclic aromatic hydrocarbons are potential threats to marine animals and human health because many of them can lead to cancer.[59] D is triphenylmethane. It can be divided into several different categories according to the substituents on the benzene ring. E is toluene. Toluene is an aromatic hydrocarbon that can cause serious social and health problems through the release of petroleum products and the spread of agricultural and industrial activities into the environment.[60] F is phenol, Phenol is an industrial organic compound that can cause gastrointestinal damage and can cause respiratory irritation, and muscle tremor.[61,62] It is very harmful to aquatic ecosystems. Phenol and phenolic wastes must be properly treated before they can be released into nature. G is a polyurethane (PU). Polyurethane is a carbamate polymer. It produces irritating gases at high temperatures. H is sodium dodecyl sulfate (SDS). It is the main raw material of detergent products, with decontamination, emulsification, and excellent foaming power. It is a kind of anionic surface-active agent with certain toxicity to the human body and is a common pollutant in urban water sources.[63]

used in microbial fuel cells for microbial power generation. *P. otitidis* AATB3 achieved the highest bioenergy (power density 280 mW/m^2, current density 800 mA/m^2), with a maximum coulomb efficiency of 15.5% at pH 7. The conclusion is that *P. otitidis* AATB4 can provide stable energy output as a novel biological battery.[70]

Generation of Polyhydroxyalkanoates

Some researchers have studied the production of PHA by *P. otitidis*. Figure 3 shows the structure and preparation process of the PHA.[71–73]

Reddy and Mohan studied the effects of medium selection and different nutrient concentrations on PHA production of *P. otitidis* isolated from sewage.[71] Some synergistic factors can improve PHA yield. An appropriate concentration of molybdenum can promote PHA production.[72,74] There is a negative correlation between enzyme specificity of coenzyme

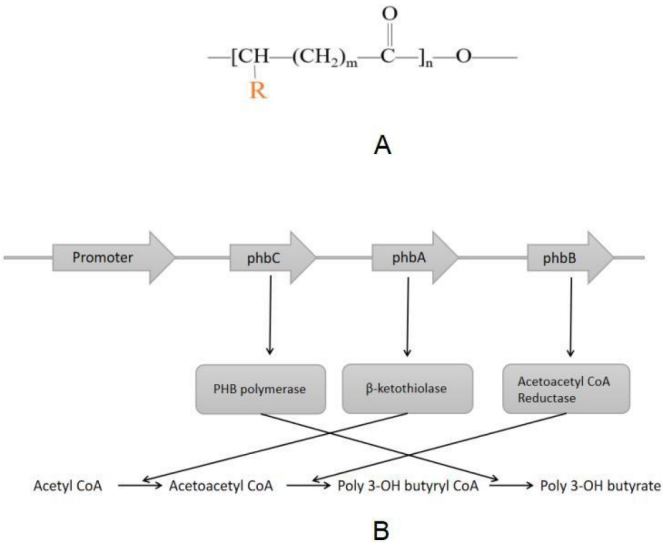

Figure 3. Chemical structure and microbial fermentation pathway of polyhydroxyalkanoates (PHA). A is the chemical structure of PHA. PHA is commonly composed of (R)-β-hydroxy fatty acids. The "R" group varies from methyl (C1) to tridecyl (C13). B is the microbial fermentation pathway of PHA. The promoter upstream of phbC transcribes the entire operon. The phbCAB operon's genes encode three enzymes (PHB polymerase, β-ketothiolase, and Acetoacetyl CoA reductase).[71] These three enzymes are involved in the synthesis of PHA. PHA is polyester produced in nature by numerous microorganisms. They can be either thermoplastic or elastomeric materials, with melting points ranging from 40°C to 180°C.[72] The mechanical properties and biocompatibility of PHA can also be changed by blending, modifying the surface, or combining PHA with other polymers and inorganic materials, making it possible for a wider range of applications. It has broad application prospects in the fields of medicine, agriculture, packaging, and electronics.[73]

and glucose-dehydrogenase 6-phosphate for PHA production in the metabolism of *P. otitidis* LFM046. Analysis revealed that the *gnd* gene, encoding 6-phosphogluconate dehydrogenase, is absent in the LFM046 genome. In LFM046, the *gnd* gene of *P. putidis* KT2440 (NAD^+ dependent) and *Escherichia coli* MG1655 ($NADP^+$ dependent) were expressed exogenous, which resulted in delayed cell growth and reduced PHA yield, respectively.[75]

The Synergy between *P. otitidis* and Plants

P. otitidis can promote plant growth and strain colony growth in some cases. Isolated *P. otitidis* and *Bacillus subtilis* strains from the rhizosphere soil of salt-tolerant plants in Pakistan could precipitate free calcium into calcium precipitation. Under salt stress, *P. otitidis* Rhizo SF7 in soil has the potential to produce acetyl-CoA carboxylase which promotes sunflower plant growth.[76] *P. otitidis* JYR27 is a rhizosphere growth bacterium resistant to phytophthora capsici and anthracnose. Researchers conducted three years of field experiments and confirmed that all selected strains significantly reduced pepper blight without affecting the rhizosphere microbial population.[77] *P. otitidis* and *P. alkaline* mixed bacteria were able to induce the expression of biofibril-related genes by corresponding to plant secretions, showing an antagonistic effect against the plant

pathogenic fungus *Rosellinia necatrix*. These two bacteria gathered, and one of their gene (*cmpA*) encodes a GGDEF/EAL domain protein. CmpA plays a role in biofilm formation, and the EAL domain is involved in this function.[78]

Antimicrobial Activity

Some researchers have studied the antagonistic effect of *P. otitidis* on microorganisms under various culture conditions. *P. otitidis* shows a resisting effect on *Naegleria fowleri* through competitive inhibition. The anti-*N. fowleri* activity of *P. otitidis* remains to be further studied.[79] Ahn studied the antibiotic production by *P. otitidis* and action mode. The researchers extracted the antibiotic from *P. otitidis* using ethyl acetate, which produced an orange halo on agar. The activity of *P. otitidis* is germicidal by reactive oxygen species.[80] *P. otitidis* with activity against *Leishmaniasis* was isolated from the midgut of Lutzomyiaevansi, an insect vector of *Leishmaniasis*.[81] *P. otitidis* isolated from cow dung was proved to have nematicidal activity against *Caenorhabditis elegans*.[82] Chellaram and Praveen isolated *P. otitidis* in short-horned grasshopper. It has antagonistic effects on *Candida albicans* and *E. coli*.[83] *P. otitidis* KAF136 (MH393230) has been proven to be a strong probiotic in biosafety, pH resistance, gastric juice resistance, bile salt resistance, and hydrophobicity solvent resistance. It was proved to resist the growth of *Aeromonas hydrophila* in aquaculture.[84] Some protozoa that can be inhibited by *P. otitidis* are shown in Figure 4.[79,85–88]

Microbial Resistance

Microbial medicine resistance is a major problem in clinical medicine and public health, which leads to an increase in medical investment and the emergence of superbugs. Many researchers have studied the resistance of *P. otitidis*, and they found a new carbapenem-resistant gene in this novel *Pseudomonas* gene, which can transcript and translate a new MBL POM-1. MBL produced by *Pseudomonas* can cause many critical diseases, such as septicemia and pneumonia.[89]

Kaur et al. studied tap water from public toilets in Punjab, India, and isolated 25 strains of bacteria, including *P. otitidis*. Drug sensitivity test results showed that cefotaxime, zoltronam, furantoline, cefepime, ceftazidime, and amoxiclaff were mostly ineffective against multiple isolates, and most of them had multiple drug resistance.[90] Nordmann et al. studied the selection medium for screening carbapenem-resistant *Pseudomonas* genus, using the medium containing meropenem (2 mg/L) to screen carbapenem-resistant *Pseudomonas*. Clinical isolates of 29 meropenem-sensitive and 56 meropenem-nonsensitive Pseudomonas strains were evaluated, the latter showing multiple carbapenem-resistance mechanisms.[91] Suzuki et al. studied the penicillin-resistant PAM gene of *P. alkaline*, and the*blaPAM-1* of *P. alcaligenes* strain MRY13-0052 was encoded in contig 73 in the chromosome without transposons or integrons around it. The results showed that *blaPAM-1* was a species-specific MBL coding gene inherent in *P. alcaligenes*. Researchers proposed that POM-1, an enzyme in *P. otitidis* is highly conservative.[92] Borgianni et al. systematically studied the biochemical characteristics of POM-1 metallo-β-lactamase from *P. otitidis*.[93]

Some researchers isolated carbapenemase-producing Gram-negative bacilli from American factories and nearby wastewater and identified 13 isolates as *P. otitidis* by the MALDI-TOF method.[94] Miyazaki et al. sequenced the whole genome of *P. otitidis* isolated from Lake Biwa, Japan, they also found a gene coding for POM, its amino acid sequence 99% identity to a similar protein found before.[19] Vieira et al. isolated meropenem-insensitive *P. otitidis* from chicken carcasses and conducted in-depth genomic characterization of the bacterium.[95] The *blaPOM-1* gene carried by *P. otitidis* K_25 encodes a MBL that is resistant to carbapenems. *P. otitidis* is the first pathogenic *Pseudomonas* to be demonstrated to constitutively express MBL and not require inducing MBL coding genes.[95] Carbapenem-resistant *P. otitidis* can be isolated from frozen food. Overexpression of the MBL coding *blaPOM-1* and *ttgABC* in *P. otitidis* is also associated with carbapenem resistance in these organisms.[96] Thaller et al. collected 20 strains of *P. otitidis* and studied the sensitivity of the metallo-β-lactams and MBL production. All strains were sensitive to piperacillin, cefotaxime, ceftazidime, and zoltronam, and occasionally showed sensitivity to carbapenems decreasing. All strains expressed MBL activity and carried a new B3 subclass MBL gene blaPOM, which was highly conserved in this species.[97] Poirel et al. studied B2 MBL, it's a PFM-like enzyme, produced by *Pseudomonas fluorescein*. It is different from B3 MBL produced by *P. otitidis*, which is *P. otitidis*' inherent MBL coding gene.[98] The prevalence and diversity of MBL coding gene containing integrons in MBL producing *Pseudomonas sp*. have been studied. Researchers investigated alleles associated with drug resistance, and analysis of blavim-2 and BLaiMP-1 alleles showed that all *Pseudomonas*, including *P. otitidis*, had BLaiMP-6.[99] Martins et al. discovered that some birds were colonized by *P. otitidis* who have *bla*POM-1 gene that encodes MBL.[100] Kim et al. isolated *P. otitidis* that produced POM-1 MBL from patients with necrotizing fasciitis and peritonitis. They made sure the important feature of *P. otitidis* is the inherent MBL coding gene *bla*POM-1.[25] Although *blaPOM-1* was inherent to the species, only 10% and 35% of isolates were resistant to imipenem and meropenem. Interestingly, the carbapenem-insensitive strains are always sensitive to piperacillin, ceftazidime, or aztreonam. This may be because the catalytic efficiency of POM-1 against carbapenems is higher than that of piperacillin.[93] Some researchers isolated 122 carbapenemase-producing bacterial strains and studied two *P. otitidis* strains' POM-1 coding gene, they confirmed that carbapenase could be produced without induction.[101]

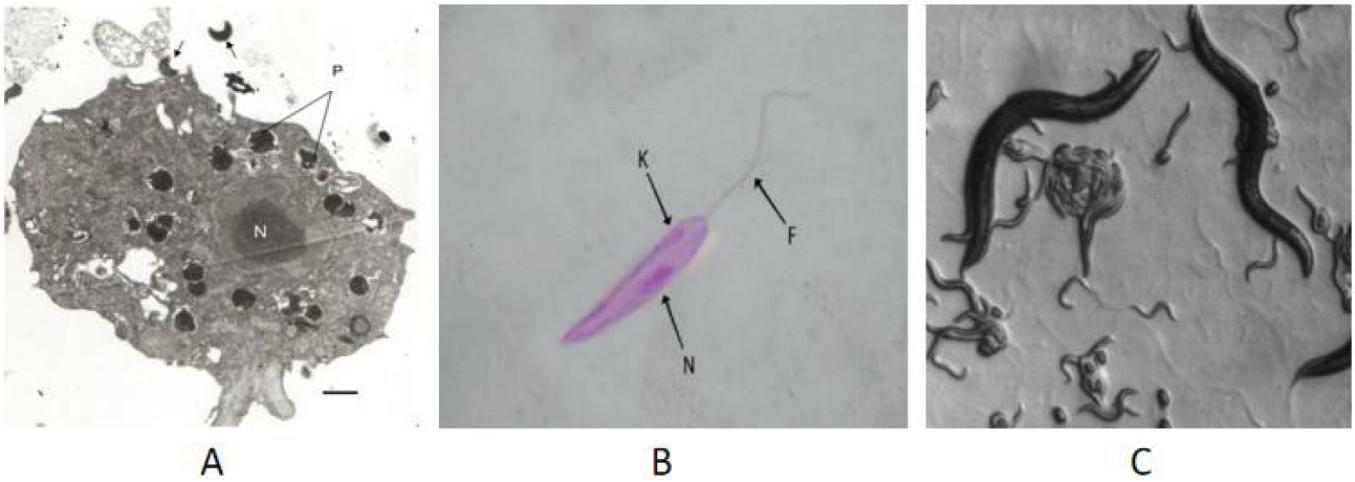

Figure 4. The comparison of three different protozoa from literature. Section A is *Naegleria fowleri*,[85] *Naegleria fowleri* can cause fatal primary amebic meningoencephalitis (PAM) in humans.[79] Section B Promastigote form of *Leishmania sp*. (N-nucleus, K-kinetoplast, F-flagellum),[86] *Leishmaniasis* is a disease caused by the bite of lutzomyia or termites. It can cause host infections in humans, rodents, and dogs.[87] Section C is *Caenorhabditis elegans*. *Caenorhabditis elegans* is a free-living nematode with relatively simple behaviors and structures. A complete life cycle takes three days. The organism is transparent throughout its life cycle, observing its structure and biological processes possible by microscopy.[88]

Usage in Farm

Researchers have studied the use of *P. otitidis* in agriculture and husbandry, such as herbicide resistance or the production of various digestive enzymes. Some researchers used herbicides as a carbon source to isolate *Pseudomonas* from three soil types cultivated corn and cucumber. It was proved that *P. otitidis* could improve soil contaminated with herbicides.[102] Lipase is a carboxyl ester hydrolase that hydrolyses triglycerides into glycerol and fatty acids. It is widely used in the food and feed processing industry. Shaini and Jayasree isolated fat-degraded bacteria WCS1C2 and WCS3C2 from compost. WCS3C2 strain had 91% homology with *P. otitidis*. And, it had a good ability to degrade fat.[103] Ramani et al. isolated a *P. otitidis* strain from sunflower seed oil waste and found that it could produce lipase and hydrolyze sunflower seed oil.[104] Fibriana et al. proposed that the biotransformation of oil-based wastes from the agricultural industry by the production of lipase in the form of solid-state fermentation or submerged fermentation would play a potential role in future biotechnology.[105] Kumar and Vyas isolated one *P. otitidis* strain that showed cellulase activity on the screening medium of carboxymethyl cellulose (CMC).[106] Huang et al studied the degradation of feather powder by isolated *P. otitidis* H11. Feather powder contains a large amount of keratin, which is difficult to hydrolysed by normal proteases to produce oligopeptides. The researchers found that under the optimized fermentation conditions, the feathers were almost completely degraded by *P. otitidis* H11. This kind of hydrolysate can be used as a supplement to feeds and as a source of extraction of functional oligopeptides.[107] Fatoni and Zusfahair isolated a thermophilic protease-producing strain *P. otitidis* WN1 from a hot spring in Indonesia. The protease was separated and partially purified.[108] Protease is one of the most important enzymes used in husbandry and industry. Compared with plant or animal tissues, microorganisms are the most studied source of proteases.[109]

Usage in Healthcare

Zearalenone is a non-steroidal estrogenic mycotoxin produced by a variety of *Fusarium* species, which can cause estrogen increase and related poisoning in livestock and humans.[110] Tan et al. isolated a strain of *P. otitidis* TH-N1 that could degrade zearalenone from the rumen of cattle and evaluated its isolation effect.[111] Uricase is an enzyme that can make uric acid oxidize rapidly, prevent uric acid from being absorbed and excreted by renal tubules, and has ameliorative effects on nodular ventilation, urinary calculus, and hyperuricemia caused by renal failure.[112] Lee et al. isolated a *P. otitidis* SN4 strain and studied the isolation, partial purification, and enzymatic properties of its product, a 33 kDa uricase.[113] Melanin has found great application in various industries due to its unique antioxidant properties.[114] Because of the increasing demand for melanin, scientific researchers are working to find more microbes that have the potential to produce melanin on a large scale. Deepthi et al. studied melanin produced by*P. otitidis* and compared the relevant tyrosinase gene sequences.[115] L-asparaginase is a very important antitumor drug widely used in the treatment of acute lymphoblastic leukemia and other malignant tumors. L-asparagine is a non-essential amino acid, which is an important nutrient for cancer cells. L-asparaginase can be used as an anticancer agent due to its high chemical efficiency in converting asparagine to ammonia and aspartic acid.[116] In the food industry, harmful by-product glutamine may be produced during the fermentation of L-asparaginase. The avoidance of glutamine production is one of the areas of current research.[117]

Shi et al. studied a pH 7.5 L-asparaginase produced by *P. otitidis*. Before this, many rhizobia genes that encode L-asparaginase have been submitted to the NCBI database. Their research is the first *P. otitidis* that can encode the L-asparaginase coding gene (*AnsA)*.[118] L-asparaginase is divided into two subtypes, L-asparaginase EI and II, according to its localization in cells.[119] The enzymatic activity of L-asparaginase obtained by fermentation of *P. otitidis* could reach 0.37 IU/ mL.[120] The maximum molecular weight of L-asparaginase produced by *P. otitidis* was 205±3 kDa.[121] The enzyme is a homologous hexamer, and the isoelectric point of the enzyme was 5.5. Purified asparaginase is non-toxic to non-cancerous FR-2 cells or human blood lymphocytes but can induce apoptosis of human leukemia MOLT-4 cells.[122]

PRODUCTS AND MECHANISMS

blaPOM-1 Gene and its Product POM-1 Metallo-β-lactamase

MBL is an enzyme that can change the spatial structure of lactam antibiotics.[123] It belongs to group 3 according to bush classification,[124] while belongs to B group according to Ambler classification.[125] The most characteristic of this enzyme group is that it can hydrolyze carbapenems and other similar antibiotics. It can bind to the β-lactam rings, causing the β-lactam ring cleavage and destruction.[126] MBL had little effect on piperacillin and amtronam. Its activity was not inhibited by clavulanic acid and other β-lactamase inhibitors, but it was inhibited by EDTA.[127] Its enzyme activity center requires the participation of metal zinc ions, so it is called MBL.[123] Carbapenems, a kind of β-lactam antibiotic, are considered the last resort for the treatment of infections caused by multidrug-resistant pathogens.[128]

The β-lactam antibiotics refer to a class of antibiotics with β-lactam ring in the chemical structure, including penicillin and cephalosporin, which are the most used in clinics, as well as other atypical β-lactam antibiotics. These antibiotics have the advantages of strong bactericidal activity, low toxicity, wide indications, and good clinical efficacy.[129] Changes in the chemical structure of this class of antibiotics especially the side chain, lead to many different antimicrobial profiles and actions, as well as a variety of clinical pharmacological properties.[130] Most kinds of β-lactam antibiotics have similar mechanisms of action. All of them can inhibit the cell wall mucin synthase, which is penicillin-binding proteins (PBPs), and thus hinder the synthesis of cell wall mucin, resulting in cell wall defect and cell swelling and cleavage. In addition, the lethal effect on bacteria should include triggering the activity of the bacteria's autolysin, so the mutant lacking autolysin shows drug resistance.[131] Humans and animals have no cell wall, not affected by β-lactam medicines, so this class of drugs has selective bactericidal effects on bacteria with low toxicity to the host.[123] The special PBPs on bacterial cell membranes are the target of β-lactam. The number, molecular weight, and sensitivity of PBPs to β-lactam antibiotics were different, but taxonomically similar bacteria have similar PBPs types and physiological functions.[131] *E. coli*, for example, has seven PBPs. PBP1A and PBP1B are associated with bacterial lengthening, while PBP2 is associated with membrane tube shape. PBP3 has the same function as PBP1A, but its quantity is small, and it is related to bacterial division. Most penicillin or cephalosporin antibiotics are mainly combined with PBP1 and/or PBP3 to form filamentous and spherical bodies, which cause deformation and atrophy of bacteria and gradually dissolve and die. PBP4, 5, and 6 are related to the activity of carboxypeptidase and have no importance to the survival and reproduction of bacteria.[132]

The genes that produce MBL can be divided into two types according to their level of activity. One is a mobile genetic element carried by plasmids that can move between different bacteria in the same ecological environment.[133–135] Most of the MBL coding genes they expressed were from shuttle plasmids. Some researchers pointed out that some pathogenic genes come from bacteriophages, which do not integrate into chromosomes when they infect bacteria but produce plasmid derivatives that can travel between individuals of different bacteria.[136] Another kind of gene that produces MBL is so conserved that it exists only in one species and cannot move between species or bacteria.[137]

Common resistance mechanisms of *Pseudomonas* include overexpression of the efflux system and AmpC B-lactamase, as well as mutation inactivation of OprD.[138] To date, *P. otitidis* is known to be associated with the endogenous MBL-POM.[139] Studies have shown that *P. otitidis* contains a *blaPOM-1* gene, which can be transcribed and translated into POM-1 enzyme, which is a B3 subgroup MBL. Some scholars have also found that the gene can be expressed without induction. POM is a tetramerase with broad substrate specificity and has higher catalytic activity against penicillin and carbapenems than cephalosporins.[97]

Thaller et al. cloned the POM gene from *P. otitidis*, and the clone producing MBL was named CT-1, carrying about 6 KB of DNA. Sequencing of the inserted fragment revealed the presence of an 859 bp open reading frame (ORF) encoding a protein like B3 subclass MBLs. It was named POM1 (named after *P. otitidis* MBL). The ORF begins with a GTG codon, preceded by an identifiable ribosomal binding site and a putative promoter region.[97] POM-1 enzyme showed the closest similarity to *Stenotrophomonas maltophilia* L1 enzyme (60%-64%), and it has low similarity with other B3 subclass enzymes.[140]

Some researchers sequenced and analyzed the upstream and downstream of the POM gene of *P. otitidis* and found that the region on it was an open reading frame for predicting the production of histamine kinase. Through gene comparison, it was found to be homologous with the PA_2882 pro-

tein gene of *P. aeruginosa*.[141] Downstream of BLAPom-1 is a phosphonate-capable operon protein homologous to *sel*D that is highly conserved.[97] Phylogenesis sketch of subclass B3 MBL enzymes and *bla*POM-1 gene are shown in Figure 5.

Mechanism of Biosurfactant Formation

Biosurfactant is a secondary metabolite synthesized by various bacteria, yeast, and filamentous fungi. It not only has the physicochemical properties of the chemical synthesis of surfactants, but also has the advantages of being non-toxic, biodegradable, and so on. It has a very broad application prospect.[142] Biosurfactant interferes with microbe-host interactions and quorum sensing mechanisms, acting as antimicrobial, insecticidal, antibiofilm, and anti-adhesive agents. Dinache et al. and Myers described the structure and principle of BS.[143,144] The molecular structure of surfactants is amphiphilic: one end is the hydrophilic group; the other end is the hydrophobic (lipophilic) group. Hydrophilic groups are often polar groups, such as carboxylic acid, sulfonic acid, sulfuric acid, amino or amine groups and their salts, hydroxyl, amide group, an ether bond, etc., it can also be used as polar hydrophilic groups. Hydrophobic groups are usually non-polar hydrocarbon chains, such as hydrocarbon chains with more than 8 carbon atoms.[144] BS produced by *P. otitidis* P4 was mainly composed of lipids and carbohydrates. The carbohydrate content was 386.25 μg/mL, and the lipid content was 0.381 mg/g.[51]

Emulsification is one of the main functions of biosurfactants produced by *P. otitidis*, as well as other kinds of biosurfactants. Emulsification refers to the uniform dispersion of one liquid in extremely small droplets in an insoluble liquid. Two insoluble liquids, such as oil and water, are divided into two layers in a container, the less dense oil on the upper layer and the denser water on the lower. If the appropriate surfactant is added under intense agitation, the oil is dispersed in water and forms an emulsion, which is called emulsification.[145] Some researchers have studied the emulsifier formed by *P. otitidis*. The formation of an emulsion is a stable interaction of hydrophobic and hydrophilic phases, largely depending on the solvent used. The biosurfactant formed by *P. otitidis* is an ideal emulsifier in a stable state.[142] Some researchers studied the emulsification of BS produced by *P. otitidis*. Diesel oil and kerosene (55%) were the most effective substrates for emulsification, while sunflower oil (45%) was the matrix with low emulsification efficiency.[146] The results show that the biosurfactants of *P. otitidis* can emulsify different types of hydrocarbon compounds and can improve the utilization rate of insoluble hydrocarbon compounds.

Researchers used different methods to detect surfactants. Ctab-methylene blue agar test is a simple method to detect anionic surfactants. Researchers found isolated *P. otitidis* strain P4 reacted positively on CTAB-methylene blue agar medium, after incubation at 37 ℃ for 48 h. A blue halo appeared around the colony.[51] The relative hydrophobicity of the bacterial surface was measured by the bacterial adhesion to the hydrocarbons method. The results showed that the hydrophobicity of the bacterial surface was up to 69.3% used crude oil for growth.[147]

Submerged fermentation of surfactant-producing *P. otitidis* was carried out to study the amount of biosurfactant produced at different fermentation stages, from the late exponential period to the end of the stationary period.[148] Ron and Rosenberg have also reported that biosurfactants are typically produced during both logarithmic and stationary phases, and that release of cell-bound surfactants into the growth medium results in a reduction in surface tension, even after the stationary phase.[149] The BS produced by *P. otitidis* P4 showed good surface tension reduction ability, reducing the surface tension of the medium from 71.18 mN/m to 33.4 mN/m.[51] This result is comparable to reports of BS production by *P. aeruginosa*.[150,151]

Different carbon and nitrogen sources have important effects on the production of biosurfactants in *P. otitidis*. The preference of microbial carbon sources for biosurfactant production depends on the strain. Different strains produce biosurfactants in different carbon sources, which may be water-soluble or insoluble substances.[152] In *P. otitidis* P4, 2% Sodium acetate was the most effective carbon source, and yeast extract (0.03%) was the most effective nitrogen source to produce biosurfactant. The growth and formation of biosurfactants were not observed in the medium without nitro.[51] *P. otitidis* cannot use ammonium ions to produce biosurfactants under certain nutritional conditions. Maneerat reported that when nitrogen concentration in the medium is depleted, the yield of surface-active compounds increases due to the decreased activity of isocitrate dehydrogenase.[153] Isocitrate dehydrogenase is catalyzed by nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) for the oxidation of isocitrate to 2-oxygen glutaric acid in the citric acid cycle. As isocitrate dehydrogenase activity declines, isocitrate and citrate begin to accumulate. In the cytoplasm, citric acid synthase converts citric acid to oxaloacetate and acetyl-coA, which act as a processor for fatty acid synthesis, thereby increasing biosurfactant production.[154]

Some researchers have found that the biosurfactant produced by *P. otitidis* is heat resistant because heating at 80-100 °C has no significant effect on surface tension and emulsifying activity.[51] However, the surfactants produced by *P. otitidis* are not adapted to all environments. Any decrease or increase in emulsifying activity at extreme temperatures may be due to some structural changes in surfactant molecules.[155] The surface tension and emulsifying activity of biosurfactants remain stable over a wide pH range (3-11).[156] The highest emulsifying activity (68.7%) was observed at neutral pH (pH=7), but significant stable emulsifying activity was also observed at acidic pH (pH=3, 44.4%) and alkaline pH (pH=11, 54.5%). The decrease in emulsification under extreme pH conditions may be due to partial precipitation of biosurfactants.[146] Biosurfactants

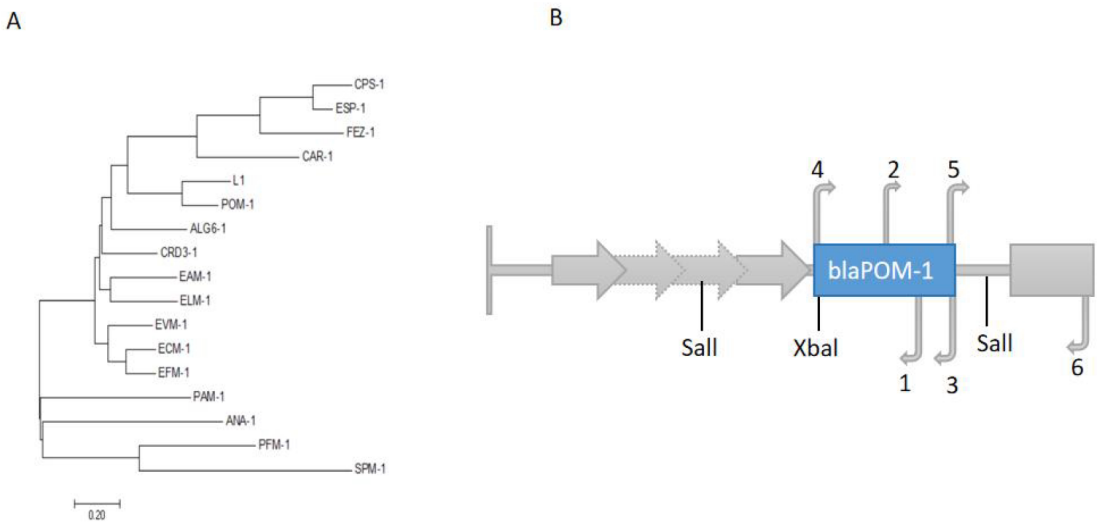

Figure 5. Phylogenesis sketch of subclass B3 MBL enzymes and *blaPOM-1* gene. A is a Phylogenesis sketch of subclass B3 MBL enzymes. The closest match to POM-1 is L1 from *Stenotrophomonas maltophilia*, their similarity is 60%-64%. B is Genetic context of *blaPOM-1*. Numbered arrows show the location of primers used for PCR amplification (Section B): 1, Pom-seq/F; 2, Pom-seq/R; 3, POTSEQ/F; 4, POM948/R; 5, CT1ft/F; 6, CT1Prom/R.[97]

can withstand 2.0% to 10.0% salt concentrations, with maximum emulsifying activity observed at 4% NaCl. Helvaci et al. explained the reasons for stable surface tension at extreme salt concentrations.[157] They describe the presence of electrolytes in the culture medium that directly affects the carboxylic groups of sugar lipids. At alkaline pH, there is a net negative charge in the solution due to the ionization of the carboxylic group. In the presence of NaCl, Na^+ ions shield negative charges in a double electric layer, resulting in the formation of a tightly packed monolayer, followed by a decrease in surface tension. In brief, stability studies showed that biosurfactants remain active at extreme temperatures, pH, and salt concentrations. What needs to be pointed out is that hemolysis, drop-collapse, oil spreading, E_{24}, BATH, and surface tension methods are used to screen the presence of biosurfactants.

CONCLUSION

Pseudomonas sp. is a widely studied bacterial species. It is a new type of *Pseudomonas sp*., which was first discovered in 2002 and was systematically studied by researchers after 2006. *P. otitidis* can be isolated from the infected site of a patient. It is pathogenic and can cause a single infection or a mixture of multiple infections. *P. otitidis* can also be isolated from natural environments, such as lakes, sludge, and contaminated environments. Research showed that *P. otitidis* can be used and decompose many substances and pollutants that exist in nature, such as crude oil, polycyclic aromatic hydrocarbons, dyes, SDS, and zearalenone. *P. otitidis* can also produce some beneficial substances that can be used, such as digestive enzymes, antibiotic substances, melanin, L-asparaginase, and so on. The unique POM MBL of *P. otitidis* has received much attention. Compared with common plasmid DNA, the POM coding gene is a conserved intrinsic gene. Functionally, it can decompose Carbapenem antibiotics such as meropenem, leading to the generation of super-resistant bacteria. *Pseudomonas*, including textitP. otitidis, can produce a large sum of biofilms. Like other bacteria, *P. otitidis* sticks to material surfaces and secretes many extracellular polymers (glycolipids) that form biofilms. Biofilm can support and protect the whole microflora in a certain area. There is also research on the synergistic effect of plants, the research on biological batteries, and the research on the production of degradable plastic PHA by *Pseudomonas*. Due to the late discovery of *P. otitidis* and the fact that *P. otitidis* is easily confused with other bacteria in species identification, some research fields on *P. otitidis* are not in-depth at present. There are many aspects of genomics, enzymology mechanisms, and clinical research worthy of further investigation. By now, the research on *P. otitidis* is still producing new results and interesting findings continuously as this strain could offer increasing benefits to humans.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- R.M., G.J.; Data Acquisition- G.J., M.H., M.S.M.; Data Analysis/Interpretation- G.J., M.H., M.S.M.; Drafting Manuscript- G.J.; Critical Revision of Manuscript- R.M.; Final Approval and Accountability- G.J., R.M., M.H., M.S.M.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

ORCID IDs of the authors

Gao Jianfeng 0000-0002-7393-6884
Rosfarizan Mohamad 0000-0001-5672-1905
Murni Halim 0000-0002-5744-2147
Mohd Shamzi Mohamed 0000-0002-9813-2161

REFERENCES

1. Palleroni NJ, Genus I. *Pseudomonas Migula* In Bergey's Manual of Systematic Bacteriology. *Springer*. 1984; 141-199.
2. Palleroni NJ, Kunisawa R, Contopoulou R, Doudoroff M. Nucleic acid homologies in the genus *Pseudomonas. Int J Syst Evol Microbiol*. 1973;23(4):333-339.
3. Kersters K, Ludwig W, Vancanneyt M, De Vos P, Gillis M, Schleifer KH. Recent changes in the classification of the *Pseudomonads*: An overview. *Syst Appl Microbiol*. 1996;19(4):465-477.
4. Romaniuk K, Krucon T, Decewicz P, Gorecki A, Dziewit L. Molecular characterization of the pA3J1 plasmid from the psychrotolerant antarctic bacterium *Pseudomonas sp*. ANT_J3. *Plasmid*. 2017;1(92):49-56.
5. Passarelli-Araujo H, Franco GR, Venancio TM. Network analysis of ten thousand genomes shed light on *Pseudomonas* diversity and classification. *Microbiol Res*. 2022;1(254):126919. doi:10.1016/j.micres.2021.126919
6. Campbell RA, Farlow J, Freyberger HR, et al. Genome Sequences of 17 Diverse *Pseudomonas aeruginosa* phages. *Microbiol Resour Announc*. 2021;10(9):e0003121. doi:10.1128/MRA.00031-21
7. Sundararajaperumal A, Murugan N, Karthick R, Harikrishnan S. Profile of pulmonary infections in renal transplant patients. *J Evid Based Med*. 2018;5(3):260-264.
8. Hillman KM, Twigley A. Aerosol EDTA to eliminate respiratory-tract *Pseudomonas. Lancet*. 1984;324(8394): 99. doi:10.1016/s0140-6736(84)90265-4
9. Beasley KL, Cristy SA, Elmassry MM, et al. During bacteremia, *Pseudomonas aeruginosa* PAO1 adapts by altering the expression of numerous virulence genes including those involved in quorum sensing. *PloS one*. 2020;15(10):e0240351. doi:10.1371/journal.pone.0240351
10. Kalpana Thalava, Vigila J. Urinary infections in pregnancy conditions. *J Pharm Res Int*. 2021;2(17):22-28.
11. Lee K, Kim CK, Yong D, et al. POM-1 metallo-β-lactamase–producing *Pseudomonas otitidis* isolate from a patient with chronic otitis media. *Diagn Microbiol Infect Dis*. 2012;72(3): 295-296.
12. Poirel L, Palmieri M, Brilhante M, Masseron A, Perreten V, Nordmann P. PFM-like enzymes are a novel family of subclass B2 metallo-β-lactamases from *Pseudomonas synxantha* belonging to the *Pseudomonas fluorescens* complex. *Antimicrob Agents Chemother*. 2020;64(2):1700-1719.
13. Silveira MC, Albano RM, Rocha-de-Souza CM, et al. Description of a novel IncP plasmid harboring blaKPC-2 recovered from a SPM-1-producing *Pseudomonas aeruginosa* from ST277. *Infect Genet Evol*. 2022;102:105302. doi:10.1016/j.meegid.2022.105302
14. Lalucat J, Gomila M, Mulet M, Zaruma A, García-Valdés E. Past, present and future of the boundaries of the *Pseudomonas* genus: Proposal of Stutzerimonas gen. Nov. *Syst Appl Microbiol*. 2022;45(1):126289. doi:10.1016/j.syapm.2021.126289
15. Olsen RH, Metcalf ES, Todd JK. Characteristics of bacteriophages attacking psychrophilic and mesophilic *Pseudomonas. J Virol*. 1968;2(4):357-364.
16. Chakrabarty AM. Gunsalus IC. Autonomous replication of a defective transducing phage in *Pseudomonas putida. Virol*. 1969;38(1):92-104.
17. Cornelis, P Ed. *Pseudomonas*: Genomics and molecular biology. *Horizon Scientific Press*; 2008.
18. Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H. Phylogenetic affiliation of the *Pseudomonas* based on 16S rRNA sequence. *Int J Syst Evol Microbiol*. 2000;50(4):1563-1589.
19. Miyazaki K, Hase E, Maruya T. Complete genome sequence of *Pseudomonas otitidis* Strain MrB4, isolated from Lake Biwa in Japan. *Microbiol Resour Announc*. 2020;9(16):e00148-20. doi:10.1128/MRA.00148-20
20. Abed, Wu H, Kareem SM. Molecular detection of *gyrA* and *mexA* genes in Pseudomonas aeruginosa. *Mol Biol Rep*. 2021;48(12):7907-7912.
21. Manivannan B, Mahalingam N, Jadhao S, Mishra A, Nilawe P, Pradeep BE. Draft genome sequence of a clinically isolated extensively drug-resistant *Pseudomonas aeruginosa* strain. *Genome Announc*. 2016;4(2):2-16.
22. Kačániová M, Klūga A, Kántor A, et al. Comparison of MALDI-TOF MS Biotyper and 16S rDNA sequencing for the identification of *Pseudomonas* species isolated from fish. *Microb Pathog*. 2019;132:313-318.
23. Roland PS, Stroman DW. Microbiology of acute otitis externa. *The Laryngoscope*. 2002;112(7):1166-1177.
24. Clark LL, Dajcs JJ, McLean CH, Bartell JG, Stroman DW. *Pseudomonas otitidis sp*. nov., isolated from patients with otic infections. *Int J Syst Evol Microbiol*. 2006;56(4):709-714.
25. Kim D, Hong SK, Seo YH, et al. Two non-otic cases of POM-1 metallo-β-lactamase-producing *Pseudomonas otitidis* infection: Necrotizing fasciitis and pan-peritonitis. *J Glob Antimicrob Resist*. 2016;7:157-158.
26. Balasubramani G, Induja K, Aiswarya D, et al. Isolation and characterization of human foot crack–associated bacterium, *Pseudomonas otitidis*, and its biological propensity. *Smart Science*. 2019;7(2):79-90.
27. Yamada K, Aoki K, Nagasawa T, et al. Complete whole-genome sequence of the novel Pseudomonas species strain TUM18999, isolated from a patient with a burn wound in Japan. *J Glob Antimicrob Resist*. 2021;24:395-397.
28. Adriana LC, Alexandros NV, Ming C, Mats L. *Pseudomonas otitidis* bacteraemia in a patient with COPD and recurrent pneumonia: Case report and literature review. *BMC Infect Dis*. 2021;21:868. doi:10.1186/s12879-021-06569-8
29. García-Ulloa M. II, Souza V, Olmedo-Álvarez G, Eguiarte LE. Can bacterial populations go extinct? Evolutionary biology and bacterial studies in Cuatro Ciénegas shed light on the extinction process. In *Conflicts Between Biodiversity Conservation and Humans*. 2022;143-162.
30. Reddy CSK, Ghai R, Kalia V. Polyhydroxyalkanoates: An overview. *Bioresour Technol*. 2003;87(2):137-146.
31. García-Ulloa MI, Escalante AE, Moreno-Letelier A, Eguiarte LE, Souza V. Evolutionary rescue of an environmental *Pseudomonas otitidis* in response to anthropogenic perturbation. *Front Microbiol*. 2021;11:563885. doi:10.3389/fmicb.2020.563885
32. Rodríguez-Verdugo A, Souza V, Eguiarte LE, Escalante AE. Diversity across seasons of culturable *Pseudomonas* from a Des-

iccation Lagoon in Cuatro Cienegas, Mexico. *Int J Microbiol.* 2012;201389. doi:10.1155/2012/201389

33. Jun SR, Wassenaar TM, Nookaew I, et al. Diversity of Pseudomonas genomes, including populus-associated isolates, as revealed by comparative genome analysis. *Appl Environ Microbiol.* 2015;82(1):375-383. doi:10.1128/AEM.02612-15
34. Dahiya S, Mohan SV. Strategic design of synthetic consortium with embedded wastewater treatment potential: Deciphering the competence of isolates from diverse microbiome. *Front Environ Sci.* 2016; 4:30. https://doi.org/10.3389/fenvs.2016.00030
35. Dasgupta D, Ghosh R, Sengupta TK. Biofilm-mediated enhanced crude oil degradation by newly isolated *Pseudomonas* species. *ISRN Biotechnol.* 2013;2013:250749. doi:10.5402/2013/250749
36. Gogoi B, Das I, Gogoi M, Charingia D, Bandyopadhyay T, Borah D. Bioremediation of motor oil-contaminated soil and water by a novel indigenous *Pseudomonas otitidis* strain DU13 and characterization of its biosurfactant. *3 Biotech.* 2022;3(12):68. doi:10.1007/s13205-022-03133-2
37. Peng YH, Shih YH, Lai YC, Liu YZ, Liu YT, Lin NC. Degradation of polyurethane by bacterium isolated from soil and assessment of polyurethanolytic activity of a *Pseudomonas putida* strain. *Environ Sci Pollut Res Int.* 2014;21(16):9529-9537.
38. Bestawy EE, El-Shatby BF, Eltaweil AS. Integration between bacterial consortium and magnetite (Fe3O4) nanoparticles for the treatment of oily industrial wastewater. *World J Microbiol Biotechnol*. 2020;36(9):141. doi:10.1007/s11274-020-02915-1
39. Mohammadi F, Sepahy A, Naghavi N, Salehi M. Removal of phenolic compounds from Spent caustic wastewater by the isolated and detected *Pseudomonas otitidis. Adv Biores.* 2017; 8(5):123-129. doi: 10.15515/abr.0976-4585.8.5.123129
40. Maitra S, Maity D, Kundu P, Adhikarinee S. Isolation and identification of a bacterial strain from soil for bioremediation of phenol for pollution control. *J Indian Chem Soc.*2020;97(4):607-612.
41. Mohanty SS, Jena HM. Biodegradation of phenol by free and immobilized cells of a novel *Pseudomonas* sp. NBM11. *Braz J Chem Eng*. 2017;34:75-84.
42. Poyraz N. Isolation of novel toluene degrading bacteria from waste water treatment plants and determination of their toluene tolerance and other biotechnological potential. *Pol J Environ Stud.* 2021;30(1):811-821.
43. Dados A, Omirou M, Demetriou K, Papastephanou C, Ioannides IM. Rapid remediation of soil heavily contaminated with hydrocarbons: A comparison of different approaches. *Ann Microbiol.* 2015;65(1):241-251.
44. Anwar MS, Kapri A, Chaudhry V, et al. Response of indigenously developed bacterial consortia in progressive degradation of polyvinyl chloride. *Protoplasma.* 2016;253(4):1023-1032.
45. Xu CB, Wang WY, Li XZ. Identification and characteristics of a phenanthrene degrading bacterium. *Acta Sci.* 2015;35(3):684-691.
46. Soberón-Chávez G, Lépine F, Déziel E. Production of rhamnolipids by *Pseudomonas aeruginosa. Appl Microbiol Biotechnol*. 2005;68(6):718-725.
47. Soberón-Chávez G, Maier RM. Biosurfactants: A general overview. *Biosurfactants*. 2011;1-11.
48. Holland PM, Rubingh DN. Mixed surfactant systems: An overview, 1992. doi:10.1021/bk-1992-0501.ch001
49. Haloi S, Sarmah S, Gogoi SB, Medhi T. Characterization of Pseudomonas sp. TMB2 produced rhamnolipids for ex-situ microbial enhanced oil recovery. *3 Biotech*. 2020;10(3):1-17.
50. Buonocore C, Tedesco P, Vitale GA, et al. Characterization of a new mixture of mono-rhamnolipids produced by *Pseudomonas gessardii* isolated from Edmonson Point (Antarctica). *Mar Drugs.* 2020;18(5): 269. doi:10.3390/md18050269
51. Singh P, Tiwary BN. Isolation and characterization of glycolipid biosurfactant produced by a *Pseudomonas otitidis* strain isolated from Chirimiri coal mines, India. *Bioresour Bioprocess.* 2016;3(1):1-16.
52. Ibrahim AG, Abd Elsalam HE. Biodegradation of anionic surfactants (SDS) by bacteria isolated from waste water in Taif Governate. *Annu Res Rev Biol*. 2018;1-13. doi: 10.9734/ARRB/2018/41436
53. Chaturvedi V, Kumar A. Diversity of culturable sodium dodecyl sulfate (SDS) degrading bacteria isolated from detergent contaminated ponds situated in Varanasi city, India. *Int Biodeterior Biodegradation.* 2011;65(7):961-971.
54. Jovčić B, BegovIć JELENA, Lozo J, Topisirović LJ, Kojić M. Dynamics of sodium dodecyl sulfate utilization and antibiotic susceptibility of strain *Pseudomonas sp.* ATCC19151. *Arch Biol Sci.* 2009;61(2):159-164.
55. Ibrahim AG, Abd Elsalam HE. Enhancement the biodegradation of sodium dodecyl sulfate by *Pseudomonas aeruginosa* and *Pseudomonas otitidis* isolated from waste water in Saudi Arabia. *Annu Res Rev Biol.* 2018; 1-7. doi: 10.9734/arrb/2018/43744
56. Jing WU, Byung-Gil JU, Kyoung-Sook KI, Young-Choon LE, Nak-Chang SU. Isolation and characterization of *Pseudomonas otitidis* WL-13 and its capacity to decolorize triphenylmethane dyes. *J Environ Sci.* 2009;21(7):960-964.
57. Jing W, Kang NY, Kim KS, Sung NC, Lee YC. Isolation and characterization of *Pseudomonas otitidis* Strain CV-1 capable of decolorizing triphenylmethane dye. *In Proceedings of the Korean Society of Life Science Conference.* 2007; 108.
58. Shah MP, Patel KA, Nair SS, Darji AM. Environmental bioremediation of dyes by *Pseudomonas aeruginosa* ETL-1 isolated from final effluent treatment plant of ankleshwar. *Am J Microbiol Res.* 2013;1(4):74-83.
59. Gamboa-Loira B, López-Carrillo L, Mar-Sánchez Y, Stern D, Cebrián ME. Epidemiologic evidence of exposure to polycyclic aromatic hydrocarbons and breast cancer: A systematic review and meta-analysis. *Chemosphere.* 2022;290:133237. doi:10.1016/j.chemosphere.2021.133237
60. Benignus VA. Health effects of toluene: A review. *Neurotoxicology.* 1981;2(3): 567-588.
61. Buckley JP, Quirós-Alcalá L, Teitelbaum SL, Calafat AM, Wolff MS, Engel SM. Associations of prenatal environmental phenol and phthalate biomarkers with respiratory and allergic diseases among children aged 6 and 7 years. *Environ Int.* 2018;115:79-88.
62. Itoh M. The role of brain acetylcholine in phenol-induced tremor in mice. *Arch Oral Biol*. 1995;40(5):365-372.
63. Reynolds JA, Tanford C. The gross conformation of protein-sodium dodecyl sulfate complexes. *J Biol Chem.* 1970;245(19):5161-5165.
64. Sleutels TH, Ter Heijne A, Buisman CJ, Hamelers HV. Bioelectrochemical systems: An outlook for practical applications. *Chem Sus Chem.* 2012;5(6):1012-1019.
65. Carver A, Gallicchio VS. Heavy metals and cancer. In *Cancer Causing Substances*. 2018. doi:10.5772/intechopen.70348.
66. Naguib MM, Khairalla AS, El-Gendy AO, Elkhatib WF. Iso-

lation and characterization of mercury-resistant bacteria from wastewater sources in Egypt. *Can J Microbiol*. 2019;65(4):308-321.

67. Yeoh CC. Production, partial purification and characterization of yellow-green fluorescent siderophores produced by *Pseudomonas otitidis* B1, UTAR, Doctoral dissertation, 2014.
68. Ai C, Hou S, Yan Z, et al. Recovery of metals from acid mine drainage by bioelectrochemical system inoculated with a novel exoelectrogen, *Pseudomonas* sp. E8. *Microorganisms*. 2019;8(1):41. doi:10.3390/microorganisms8010041
69. Modestra, J.A. and Mohan, S.V. Bio-electrocatalyzed electron efflux in Gram-positive and Gram-negative bacteria: An insight into disparity in electron transfer kinetics. *RSC Adv*. 2014;4(64):34045-34055.
70. Thulasinathan B, Nainamohamed S, Samuel JO, et al. Comparative study on *Cronobacter sakazakii* and *Pseudomonas otitidis* isolated from septic tank wastewater in microbial fuel cell for bioelectricity generation. *Fuel*. 2019;248:47-55.
71. Reddy MV, Mohan SV. Effect of substrate load and nutrients concentration on the polyhydroxyalkanoates (PHA) production using mixed consortia through wastewater treatment. *Bioresour Technol*. 2012;114:573-582.
72. Reddy MV, Nikhil GN, Mohan SV, Swamy YV, Sarma PN. *Pseudomonas otitidis* as a potential biocatalyst for polyhydroxyalkanoates (PHA) synthesis using synthetic wastewater and acidogenic effluents. *Bioresour Technol*. 2012; 123 471-479.
73. Wang Y, Yin J, Chen GQ. Polyhydroxyalkanoates, challenges and opportunities. *Curr Opin Biotechnol*. 2014;30:59-65.
74. Venkateswar Reddy M, Chitanya DNSK, Nikhil GN, Venkata Mohan S, Sarma PN. Influence of Co-F actor on enhancement of bioplastic production through wastewater treatment. *Clean–Soil Air Water*. 2014;42(6):809-814. https://doi.org/10.1002/clen.201300105
75. Cardinali-Rezende J, Di Genova A, Nahat RA, et al. The relevance of enzyme specificity for coenzymes and the presence of 6-phosphogluconate dehydrogenase for polyhydroxyalkanoates production in the metabolism of *Pseudomonas sp*. LFM046. *Int J Biol Macromol*. 2020;163:240-250.
76. Idrees N, Liaqat I, Qurashi AW. *Pseudomonas otitidis* and *Bacillus subtilis* from saline soil of Pakistan exhibiting a potential for calcium precipitation. *Inter J Econ Envir Geo*. 2018;9(4):62-67.
77. Sang MK, Shrestha A, Kim DY, Park K, Pak CH, Kim KD. Biocontrol of Phytophthora blight and anthracnose in pepper by sequentially selected antagonistic rhizobacteria against *Phytophthora capsici. Plant Pathol J*. 2013;29(2):154-167.
78. Pintado A, Pérez-Martínez I, Aragón IM, et al. The Rhizobacterium *Pseudomonas alcaligenes* AVO110 induces the expression of biofilm-related genes in response to Rosellinia necatrix exudates. *Microorganisms*. 2021;9(7):1388. doi:10.3390/microorganisms9071388
79. Ruenchit P, Whangviboonkij N, Sawasdipokin H, Phumisantiphong U, Chaicumpa WA. Search for anti-Naegleria fowleri agents based on competitive exclusion behavior of microorganisms in natural aquatic environments. *Pathogens*. 2021;10(2):142. doi:10.3390/pathogens10020142
80. Ahn KJ. Antibiotic production of *Pseudomonas otitidis* PS and mode of action. *Biotechnol Lett*. 2018;46(1):40-44.
81. Vivero RJ, Mesa GB, Robledo SM, Herrera CXM, Cadavid-Restrepo G. Enzymatic, antimicrobial, and leishmanicidal bioactivity of gram-negative bacteria strains from the midgut of *Lutzomyia evansi*, an insect vector of Leishmaniasis in Colombia. *Biotechnol Rep (Amst)*. 2019;24:e00379 doi:10.1016/j.btre.2019.e00379
82. Lu H, Wang X, Zhang K, Xu Y, Zhou L, Li G. Identification and nematicidal activity of bacteria isolated from cow dung. *Ann Microbiol*. 2014;64(1):407-411.
83. Chellaram C, Praveen MM. Molecular characterization of antagonistic bacteria, *Pseudomonas otitidis* from Insect gut, short horned grasshopper. *J Pure Appl Microbiol*. 2015;9(23):91-96.
84. Husain F, Duraisamy S, Balakrishnan S, Ranjith S, Chidambaram P, Kumarasamy A. Phenotypic assessment of safety and probiotic potential of native isolates from marine fish *Moolgarda seheli* towards sustainable aquaculture. *Biologia (Bratisl)*. 2022;77(3):775-790.
85. Siddiqui R, Ali IK, Cope JR, Khan NA. Biology and pathogenesis of *Naegleria fowleri. Acta Tropica*. 2016;164(3):75-94.
86. Al-Mamary NI, Al-Hayali HL. Effect of synergism of thalidomide and liposomal amphotericin-B on *Leishmania tropica* and *Leishmania donovani* promastigote. *Revis Bionatura*. 2022; 7(2): 59. doi:10.21931/RB/2022.07.02.59
87. Desjeux P. Leishmaniasis: Current situation and new perspectives. *Comp Immunol Microbiol Infect Dis*. 2004;27(5):305-318.
88. Ankeny RA. The natural history of *Caenorhabditis elegans* research. *Nat Rev Genet*. 2001;2(6):474-479.
89. Wang W, Wang X. Prevalence of metallo-β-lactamase genes among *Pseudomonas aeruginosa* isolated from various clinical samples in China. *Lab Med*. 2020;44(4):97-203.
90. Kaur R, Singh D, Kesavan AK, Kaur R. Molecular characterization and antimicrobial susceptibility of bacterial isolates present in tap water of public toilets. *Int Health*. 2020;12(5):472-483.
91. Nordmann P, Fournier C, Poirel L. A Selective culture medium for screening carbapenem resistance in *Pseudomonas* spp. *Microb Drug Resist*. 2021;27(10): 1355-1359.
92. Suzuki M, Suzuki S, Matsui M, Hiraki Y, Kawano F, Shibayama K. A subclass B3 metallo-β-lactamase found in *Pseudomonas alcaligenes*. *J Antimicrob Chemother*. 2014;69(5):1430-1432.
93. Borgianni L, De Luca F, Thaller MC, Chong Y, Rossolini GM, Docquier JD. Biochemical characterization of the POM-1 metallo-β-lactamase from *Pseudomonas otitidis. Antimicrob Agents Chemother*. 2015; 59(3):1755-1758.
94. Mathys DA, Mollenkopf DF, Feicht SM, et al. Carbapenemase-producing *Enterobacteriaceae* and *Aeromonas* spp. present in wastewater treatment plant effluent and nearby surface waters in the US. *PloS One* 2019;14(6):e0218650. doi:10.1371/journal.pone.0218650
95. Vieira TR, Sambrano GE, Silva NM, et al. In-Depth genomic characterization of a meropenem-nonsusceptible *Pseudomonas otitidis* strain contaminating chicken carcass. *Acta Sci Vet*. 2020;48. doi:10.22456/1679-9216.103176
96. Wong MHY, Chi Chan EW, Chen S. Isolation of carbapenem-resistant *Pseudomonas* spp. from food. *J Glob Antimicrob Resist* 2015;3(2):109-114.
97. Thaller MC, Borgianni L, Di Lallo G, et al. Metallo-β-lactamase production by *Pseudomonas otitidis*: A species-related trait. *Antimicrob Agents Chemother*. 2011;55(1):118-123.
98. Poirel L, Palmieri M, Brilhante M, Masseron A, Perreten V, Nordmann P. PFM-like enzymes are a novel family of subclass B2 metallo-β-lactamases from *Pseudomonas synxantha* belonging to the *Pseudomonas fluorescens* complex. *Antimicrob Agents Chemother*. 2020;64(2):17-19.
99. Yum JH. Prevalence and diversity of MBL gene-containing inte-

grons in metallo-β-lactamase (MBL)-producing *Pseudomonas spp*. isolates disseminated in a Korean hospital. *Biomed Sci Lett.* 2019;25(4):321-330.

100. Martins WM, Narciso AC, Cayo R, et al. SPM-1-producing *Pseudomonas aeruginosa* ST277 clone recovered from microbiota of migratory birds. *Diagn Microbiol Infect Dis*. 2018; 90(3):221-227.
101. Le Terrier C, Masseron A, Uwaezuoke NS, et al. Wide spread of carbapenemase-producing bacterial isolates in a Nigerian environment. *J Glob Antimicrob Resist*. 2020;21:321-323.
102. El-Bestawy E, Sabir J, Mansy AH, Zabermawi N. Isolation, identification and acclimatization of Atrazine-resistant soil bacteria. *Ann Agric Sci*. 2013; 58(2): 119-130.
103. Shaini VP, Jayasree S. Biochemical characterization and 16S rDNA sequencing of lipolytic bacterial isolates WCS. *Natl Sci*. 2015;11(1):82-90.
104. Ramani K, Saranya P, Jain SC, Sekaran G. Lipase from marine strain using cooked sunflower oil waste: Production optimization and application for hydrolysis and thermodynamic studies. *Bioprocess Biosyst Eng*. 2013;36(3): 301-315.
105. Fibriana F, Upaichit A, Cheirsilp B June. Turning waste into valuable products: Utilization of agroindustrial oily wastes as the low-cost media for microbial lipase production. *In Journal of Physics: Conference Series*. IOP Publishing. 2021;1918(5):28-52.
106. Kumar A, Vyas P. Biochemical and molecular characterization of cellulase producing bacterial isolates from cattle dung samples. *J Adv Res Biotechnol*. 2018;1-6. doi:10.15226/2475-4714/3/1/00132.
107. Huang Y, Liu X, Ran Y, Cao Q, Zhang A, Li D. Production of feather oligopeptides by a newly isolated bacterium *Pseudomonas otitis* H11. *Poult Sci*. 2019.
108. Fatoni A, Zusfahair. Isolation and partial purification of new protease form thermophilic bacteria *Pseudomonas otitidis* WN 1 obtained from Indonesian hot spring. *Conference Paper*; 2016; Jakarta.
109. Shamsi TN, Parveen R, Fatima S. Characterization, biomedical and agricultural applications of protease inhibitors: A review. *Int J Biol Macromol*. 2016;91:1120-1133.
110. Viera-Limón MJ, Morlett-Chávez JA, Sierra-Rivera CA, Luque-Contreras D, Zugasti-Cruz A. Zearalenone induced cytotoxicity and oxidative stress in human peripheral blood leukocytes. *Toxicology*. 2015;1(1):102. doi:10.4172/2476-2067.1000102
111. Tan H, Zhang Z, Hu Y, et al. Isolation and characterization of *Pseudomonas otitidis* TH-N1 capable of degrading Zearalenone. *Food Control*. 2015;47:285-290.
112. Mahler HR, Hubscher Georg, Baum H. Studies on uricase. *J Biol Chem*. 1955; 216:625-641.
113. Lee NSIS, Khosravi HM, Ibrahim N, Shahir S. Isolation, partial purification and characterization of thermophilic uricase from *Pseudomonas otitidis* strain SN4. *Malays J Microbiol*. 2015;352-357.
114. Lerner AB, Fitzpatrick TB. Biochemistry of melanin formation. *Physiol Rev*. 1950;30(1):91-126.
115. Deepthi SS, Reddy MK, Mishra N, Agsar D. Melanin production by *Pseudomonas* sp. and *in silico* comparative analysis of tyrosinase gene sequences. *BioTechnologia (Pozn)*. 2021;102(4):411-424.
116. Shrivastava A, Khan AA, Khurshid M, Kalam MA, Jain SK, Singhal PK. Recent developments in L-asparaginase discovery and its potential as anticancer agent. *Crit Rev Oncol Hematol*. 2016;100:1-10.
117. Ehsanipour EA, Sheng X, Behan JW, et al. Adipocytes cause leukemia cell resistance to L-asparaginase via release of glutamine. *Cancer Res*. 2013;73(10): 2998-3006.
118. Shi R, Liu Y, Mu Q, Jiang Z, Yang S. Biochemical characterization of a novel L-asparaginase from *Paenibacillus barengoltzii* being suitable for acrylamide reduction in potato chips and mooncakes. *Int J Biol Macromol*. 2017;96:93-99.
119. Ghosh S, Chaganti SR, Prakasham RS. Polyaniline nanofiber as a novel immobilization matrix for the anti-leukemia enzyme l-asparaginase. *J Mol Catal B Enzym*. 2012;74(1-2):132-137.
120. Sharma A, Husain I. Evaluation of antitumor activity of glutaminase-free periplasmic asparaginase from indigenous bacterial isolates as candidates for cancer therapy. P*roc Natl Acad Sci India Sect B Biol Sci.* 2017;87(3):997-1004.
121. Muneer F, Siddique MH, Azeem F, et al. Microbial L-asparaginase: Purification, characterization and applications. *Arch Microbiol*. 2020;202(5):967-981.
122. Husain I, Sharma A, Kumar S, Malik F. Purification and characterization of glutaminase free asparaginase from *Pseudomonas otitidis*: Induce apoptosis in human leukemia MOLT-4 cells. *Biochimie*. 2016;121:38-51.
123. Wang Z, Fast W, Valentine AM, Benkovic SJ. Metallo-β-lactamase: Structure and mechanism. *Curr Opin Chem Biol*. 1999;3(5):614-622.
124. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*. 1995;39(6):1211-1233.
125. Hall BG, Barlow M. Revised ambler classification of β-lactamases. *J Antimicrob Chemother*. 2005;55(6):1050-1051.
126. Khan AU, Maryam L, Zarrilli R. Structure, genetics and worldwide spread of New Delhi metallo-β-lactamase (NDM): A threat to public health. *BMC Microbiol*. 2017;17(1):1-12.
127. Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR. Molecular characterization of a β-lactamase gene, bla GIM-1, encoding a new subclass of metallo-β-lactamase. *Antimicrob Agents Chemother*. 2004;48(12):4654-4661.
128. Breilh D, Texier-Maugein J, Allaouchiche B, Saux MC, Boselli, E. Carbapenems. *J Chemother*. 2013;25(1):1-17.
129. Demain AL, Elander RP. The β-lactam antibiotics: Past, present, and future. *Antonie Van Leeuwenhoek*. 1999;75(1):5-19.
130. Hou JP, Poole JW. β-lactam antibiotics: Their physicochemical properties and biological activities in relation to structure. *J Pharm Sci*. 1971;60(4):503-532.
131. Zapun A, Contreras-Martel C, Vernet T. Penicillin-binding proteins and β-lactam resistance. *FEMS Microbiol Rev*. 2008;32(2):361-385.
132. De Sousa Borges A, de Keyzer J, Driessen AJ, Scheffers DJ. The *Escherichia coli* membrane protein insertase YidC assists in the biogenesis of penicillin binding proteins. *J Bacteriol*. 2015;97(8):1444-1450.
133. Yano H, Kuga A, Okamoto R, Kitasato H, Kobayashi T, Inoue M. Plasmid-encoded metallo-β-lactamase (IMP-6) conferring resistance to carbapenems, especially meropenem. *Antimicrob Agents Chemother*. 2001;45(5):1343-1348.
134. Miriagou V, Tzelepi E, Gianneli D, Tzouvelekis LS. *Escherichia coli* with a self-transferable, multiresistant plasmid coding for metallo-β-lactamase VIM-1. *Antimicrob Agents Chemother*. 2003;47(1):395-397.

135. Poirel L, Naas T, Nicolas D, et al. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob Agents Chemother.* 2000;44(4):891-897.

136. Holloway BW, Krishnapillai V, Stanisich V. *Pseudomonas* genetics. *Annu Rev Genet*. 1971;5(1):425-446.

137. Yang Z, Liu W, Cui Q, et al. Prevalence and detection of *Stenotrophomonas maltophilia* carrying metallo-β-lactamase blaL1 in Beijing, China. *Front Microbiol*. 2014;5:692. doi:10.3389/fmicb.2014.00692

138. Quale J, Bratu S, Gupta J, Landman D. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. *Antimicrob Agents Chemother*. 2006;50(5):1633-1641.

139. Lee K, Lim JB, Yum JH, et al. bla VIM-2 cassette-containing novel integrons in metallo-β-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. *Antimicrob Agents Chemother*. 2002;46(4):1053-1058.

140. Tickler IA, Shettima SA, Dela Cruz CM, et al. Characterization of carbapenem-resistant gram-negative bacterial isolates from Nigeria by whole genome sequencing. *Diagn Microbiol Infect Dis*. 2021;101(1):115422. doi:10.1016/j.diagmicrobio.2021.115422

141. Frisk A, Schurr JR, Wang G, et al. Transcriptome analysis of *Pseudomonas aeruginosa* after interaction with human airway epithelial cells. *Infect Immun*. 2004;72(9):5433-5438.

142. Reis RS, Pacheco GJ, Pereira AG, Freire DMG. Biosurfactants: Production and Applications [Internet]. Biodegradation-Life of Science. InTech; 2013. Available from: http://dx.doi.org/10.5772/56144)

143. Dinache A, Pascu ML, Smarandache A. Spectral properties of foams and emulsions. *Molecules*. 2021;26(24):7704. doi:10.3390/molecules26247704

144. Myers D. Surfactant science and technology. *Edition, John Wiley & Sons, Inc., Hoboken, NJ*. 2020.

145. McClements DJ, Gumus CE. Natural emulsifiers biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Adv Colloid Interface Sci*. 2016;234:3-26.

146. Abouseoud M, Maachi R, Amrane A, Boudergua S, Nabi A. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens. Desalination*. 2008;223(1-3):143-151.

147. Thavasi R, Sharma S, Jayalakshmi S. Evaluation of screening methods for the isolation of biosurfactant producing marine bacteria. *J Pet Environ Biotechnol S.* 2011; 1(2): 1-6.

148. Chen CY, Baker SC, Darton RC. Batch production of biosurfactant with foam fractionation. *J Chem Technol*. 2006;81(12):1923-1931.

149. Ron EZ, Rosenberg E. Biosurfactants and oil bioremediation. *Curr Opin Biotechnol*. 2002;13(3):249-252.

150. Mendes AN, Filgueiras LA, Pinto JC, Nele M. Physicochemical properties of rhamnolipid biosurfactant from *Pseudomonas aeruginosa* PA1 to applications in microemulsions. *J Biomater Nanobiotechnol*. 2015;6:64-79.

151. Gudiña EJ, Rangarajan V, Sen R, Rodrigues LR. Potential therapeutic applications of biosurfactants. *Trends Pharmacol Sci*. 2013;34(12):667-675.

152. Dubey K, Juwarkar A. Distillery and curd whey wastes as viable alternative sources for biosurfactant production. *World J Microbiol Biotechnol*. 2001;17(1):61-69.

153. Maneerat S. Biosurfactants from marine microorganisms. *Songklanakarin J Sci Technol*. 2005;27(6):1263-1272.

154. Ray S, Ray, M. Purification and characterization of NAD and NADP-linked alpha-ketoaldehyde dehydrogenases involved in catalyzing the oxidation of methylglyoxal to pyruvate. *J Biol Chem*. 1982;257(18):10566-10570.

155. Aparna A, Srinikethan G, Smitha H. Production and characterization of biosurfactant produced by a novel *Pseudomonas sp*. 2B. *Colloids Surf*. B. 2012; 95:23-29.

156. Ishigami Y, Gama Y, Nagahora H, Yamaguchi M, Nakahara H, Kamata T. The pH-sensitive conversion of molecular aggregates of rhamnolipid biosurfactant. *Chem Lett*. 1987;16(5):763-766.

157. Helvacı ŞŞ, Peker S, Özdemir G. Effect of electrolytes on the surface behavior of rhamnolipids R1 and R2. *Colloids Surf*. B. 2004; 35(3-4):225-233.

How cite this article

Jianfeng G, Mohamad R, Halim M, Mohamed MS. *Pseudomonas otitidis*: Discovery, Mechanisms and Potential Biotechnological Applications. Eur J Biol 2023; 82(2): 224–238. DOI:10.26650/EurJBiol.2023.1247822