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Isolate of Catalase Enzyme-Producing Endophytic Bacteria from the Medicinal Plant Group: A Review

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Abstract:
Background*: Medicinal plants have therapeutic properties that synergize with endophytic bacteria. Medicinal plants can overcome abiotic and biotic stress by producing secondary metabolites. The occurrence of abiotic stress produces ROS, to overcome is assisted by the enzyme catalase. The presence of endophytic bacteria can make the isolation of the catalase enzyme effective because of the short time and the results obtained on a large scale. This article review aims to determine the colonization, isolation, and identification of endophytic bacteria from medicinal plants as producers of catalase enzymes.*
Materials and Methods*: The literature review uses primary literature derived from original articles published in Scopus indexed scientific journals, Web of Science (WoS), DOAJ, EBSCO, and Google Scholar. A total of 63 articles were obtained, 8 of them as main references.*
Results*: Based on the results of a literature review, endophytic bacteria have colonized the roots, stems, leaves, flowers, and seeds of Thymus vulgaris, Curcuma longa, Panax ginseng, Aloe vera, and various other medicinal plants. Isolation of the catalase enzyme was carried out by extracting the enzyme using ultrasonication, then purified by anion exchange chromatography and purification quantification by the Bradford method. Most of the endophytic bacterial species identified were Bacillus sp. with positive catalase activity.*
Key Word*:Catalase; Endophytic bacteria; Medicinal plants*

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I. Introduction

Medicinal plants have been used by the wider community as herbal treatments because they contain various bioactive compounds with curative properties, in addition to being precursors for drug production or development.[1], [2].Medicinal plants have therapeutic properties that synergize with endophytic bacteria and are the best choice in the examination of endophytic bacteria[3], [4]. Endophytic bacteria are bacteria that live in plant tissues producing bioactive compounds and have the same characteristics as their host plants[5]. Medicinal plants act in a certain way when selecting endophytes, because they are based on the secondary metabolites produced by the plant and the composition of the root exudates[6]. Medicinal plants can combat salinity stress conditions by producing secondary metabolites that play a role in the interaction between plants and the environment for adaptation, adaptation, and defense[1]. This ability is carried out in the presence of endophytic bacteria that provide direct benefits, namely increasing nutrient uptake and modulating growth/stress-related phytohormones, such as modulating phytohormonesauxin, ethylene (ACC deaminase), cytokinins, and gibberellins [7]. Then endophytic bacteria can indirectly inhibit pests/pathogens, thereby affecting plant survival such as antibiosis (phytopathogens, pesticides), lytic enzymes (chitinases, proteases), nutritional composition (siderophores), and Induced Systemic Resistance (ISR)[7]–[9]. Examples of endophytic bacteria on their host plants include *Bacillus sp.* on the plant *Thymus vulgaris*[9], *Enterobacter sp. on the root of the aloe vera*plant[10],*B. cereus, Pseudomonas putida, Clavibactermichiganensis on turmeric (Curcuma longa)* with *positive* catalase activity[11], *B. amyloliquefaciens*in Asian ginseng *(Panax ginseng)*[12], *Kocuriarhizophila and Cronobactersakazakii*are useful in relieving abiotic stress on wheat plants[13]. Endophytic bacteria have the advantage of having a shorter life cycle compared to their host, so they are very effective for use as a medium for large-scale enzyme production due to short harvest times and do not require large areas of land. [14].

Free radicals or Reactive Oxygen Species (ROS) are a by-product of natural metabolic processes[15]. ROS produced by cells with normal metabolism reacts chemically with cellular biomolecules (nucleic acids, proteins, and lipids) causing damage to cellular activity[16]. Antioxidant defense mechanisms (as metabolites, vitamins, and enzymes) carried out by cells can reduce the harmful effects of the reactive species produced[16]. Catalase plays an important role in inhibiting the formation of ROS by breaking down hydrogen peroxide (H_2O_2) into oxygen (O_2) and water (H_2O).[17]. In addition, catalase is used as an indicator of plant antioxidant capacity, because it can detoxify ROS produced by abiotic stress.[18]. In general, catalase plays a role in dealing with stress conditions in plants, one of which is oxidative stress[19].

Catalase can be found in a variety of living organisms. Monofunctionalcatalase is mostly found in aerobic organisms[20]. Catalaseperoxidase is usually found in fungi, archaea, and bacteria, while manganese catalase is usually found only in bacteria.[21]. Catalase in bacteria functions to break down hydrogen peroxide into water and oxygen[13]. Because bacteria under certain conditions produce H_2O_2 which interferes with the metabolic system, if not decomposed the bacteria will experience death[22]. Catalase is a hemoprotein consisting of four heme groups that react with peroxide compounds so that toxic substances (H_2O_2) can be broken down into oxygen and water[21].Currently, many endophytic bacteria are used in the production of catalase such as *Bacillus sp.* has a variety of catalase with good enzymatic characteristics[23]. One species of *Bacillus* such as *Bacillus pumilus* can produce catalase with the high catalytic activity of 55,784 U/mg through recombinant enzymes expressed in *B. subtilis 168*[24]. Based on the above background, a review of articles has been carried out discussing the isolates and characteristics of endophytic bacteria that produce catalase enzymes from the medicinal plant group.

II. Material And Methods

The literature review uses primary literature derived from original articles published in indexed scientific journals Scopus, Web of Science (WoS), DOAJ, EBSCO, and Google Scholar based on keywords including isolation of endophytic bacteria, isolation of catalase enzyme, and characterization of the enzyme catalase. Judging from the endophytic bacteria that host medicinal plants, the isolation and characterization methods used. After that, the catalase enzyme activity, isolation, and characteristics of the catalase enzyme were tested. This can determine the type or species of endophytic bacteria that have the potential for the activity of the catalase enzyme they produce. A total of 63 articles were obtained, 8 of them as main references.

III. Result

The results of the literature review show that endophytic bacteria can be isolated from various types of medicinal plants and the bioactive compounds they produce. However, only certain medicinal plants can produce catalase enzyme-producing endophytic bacteria. Here are some catalase enzyme-producing endophytic bacteria from medicinal plants that have positive catalase activity, in the table below:

Table no 1: Endophytic bacteria isolated from medicinal plants and their catalase activity.

Medicinal Plant Name	Endophytic Bacteria	Bioactive Compound	Catalase Activity	References
Alhagimaurorum *Menthaspicata* *Solanumnigrum* *Achiellamillefolium* *Artemisia vulgaris* *Astraglusgossypinus*	*B. pumilus* *B. safensis* *B. safensis* *B. safensis* *B. pumilus, B. safensis* *B. pumilus*	Antifungal activity (*Penicilliumcitrinum)*	Positive catalase activity	[3]
Thymus vulgaris	*Bacilllus, Micobacterium, Enterobacter, Streptomyces, Rhodococcus, Klebsiella, Arthobacter, Escherichia, Micrococcus, Shigella, Kocuria,dietzia, Lysinibacillus, Blastococus, Cellulosimicrobium, Pseudomonas, Micromonospora*	*Bacillus sp* had the highest antagonistic activity against *Fusariumoxysporum, Fulviafulva*, and *Alternariasolani*.	Catalase activity with endophytic bacteria inoculation can reduce salinity stress in plants.	[9]
Stachyslavandulifolia	*Amycolatopsistolypophora*	Broad-spectrum antibacterial activity	-	[25]
Ocimum sanctum *Aeglemarmelos* *Calotropisprocera* *Rawolfiaserpentina* *Azadirachtaindica*	6 isolat 4 isolat 21 isolat 4 isolat 3 isolat	From 38 isolates, 15 isolates had antibacterial activity	From 38 isolates, 35 isolates (92.10%) had positive catalase activity	[26]
Panax ginseng	*Bacillus amyloliquefaciens*	Inhibition of growth of pathogenic fungi (Fusariumoxysporum)	Positive catalase activity	[12]
Tinosporacordifolia	Terdapat 38 isolat, 31 spesies*Bacillus*, 3 spesies*Pseudomonas*, dan 4 spesies*Aneurinibacillus*	Antibacterial and antifungal activity	-	[27]
Aloe vera	*Enterobacter sp.,*	antifungal activity	-	[10]

	Enterobacterasburiae, Enterobactertabaci, Enterobacterludwigii, Pantoeaagglomerans, Pectobacteriumcypripedii, Lelliottianimipressuralis, Paraburkholderia sp., Bacillus megaterium, Bacillus agri, Lysinibacillusxylanilyticus, L. macrolides, Microbacteriumaerolatum, Chryseobactriumtaiwanense			
Curcuma longa	*Bacillus cereus* *Bacillus thurigiensis* *Bacilllus sp.* *Bacillus pumilus* *Pseudomonas putida* *Clavibactermichiganensis*	Antifungal and antibacterial activity	positive negative positive positive positive positive	[11]

IV. Discussion

IV.1 Endophytic Bacteria

IV.1.1 Plant Parts Where Endophytic Bacteria Host

Endophytic bacteria are bacteria that live in plant tissues and can increase plant growth under normal conditions and stress conditions such as oxidative stress without harming the host.[3], [22], [27]. Endophytic bacteria have a life cycle mostly in the host plant which is generally located in the intracellular and intercellular parts of the vascular tissue that can be found in the roots.[10], [28]–[30]. In addition, endophytic bacteria enter the host through the zone of roots, stems, leaves, flowers, and seeds[1], [26], [27], [31]. In roots, endophytic bacteria infect plant tissues systemically, because the host plant can produce exudate on the roots significantly which can affect the endophytic bacterial group in the rhizosphere[7], [32], [33]. Colonization of endophytic bacteria is usually achieved through seeds, vegetative parts, and rhizosphere, followed by rhizoplane (root surface) and inner root colonization[1], [7], [32].

IV.1.2The Interaction of Endophytic Bacteria with Plants Produces Bioactive Compounds Similar to Their Hosts

The interaction of endophytic bacteria with their host plants is characterized by a symbiotic relationship of mutualism because both provide mutual benefits[1], [29]. Host plants provide a protective space for endophytic bacteria, produce metabolites that can increase nutrient absorption, then affect plant growth and biomass gain[30], [34]. Metabolites induce the process of plant resistance to pathogens and reduce infection with pathogens that act as disease controllers[30], [34]. The interaction between endophytic bacteria and host plants involves a coevolutionary process that is regulated by colonization, influenced by genotype, growth stage, physiological status, plant tissue type, and environmental conditions such as temperature, water, and nutrients[5], [30]. The ability of endophytic bacteria to produce the same bioactive compounds as their host plants as a result of coevolution and gene transfer (genetic recombinant)[5], [14]. Coevolution is the survival of endophytic bacteria in their host plants by breaking down plant metabolites and obtaining nutrients and energy[5], [26], [35]. Therefore, endophytic bacteria adapt to a special microenvironment by taking plant genomes through genetic variation, one of which is gene transfer[26], [35]. This evolutionary process results in a close relationship between endophytic bacteria and their host, besides that the interaction is caused by changes at the cellular and molecular levels that interfere with plant development[30], [36], [37].

Endophytic bacteria act directly on plant development and growth through biological nitrogen fixation, dissolution of phosphorus, production of phytohormones which provide resistance to biotic factors and help plants obtain nutrients [29], [30], [38]. Indirectly increasing plant growth by preventing phytopathogens through the production of antibiotics, hydrolytic enzymes that can prevent the colonization of pathogenic microbes, production of siderophores, Induced Systemic Resistance (ISR) which can release metabolites and activate plant defense mechanisms against other pathogens[26], [32].

IV.1.3 Endophytic Bacterial Colonization Process in Host Plants

Plant Growth Promoting Bacteria (PGPB), or Plant Growth Promoting Rhizobacteria (PGPR) can colonize the inside of plants and can develop as endophytic bacteria[13], [30], [39], [40]. PGPB can form endophytic bacterial populations in various plant tissues and organs[13], [30]. Colonization of endophytic bacteria in host plants is seen from several factors including endophytictaxon and strain type, plant tissue type, plant genetic composition, and biotic and abiotic environmental conditions[1], [41].

Colonization of endophytic bacteria is usually achieved through seeds, vegetative parts, and the surrounding environment (rhizoplane and phyllosphere), then explore potential entry points to access plant internal tissues[1], [7], [31], [32]. The main entrances for endophytic bacteria to enter the host plant are through root hairs or lateral roots that arise as well as stomata, wounds, and hydathodes on shoots[32], [41], [42]. Endophytic bacteria can take advantage of natural discontinuities in plants to access plant internal tissues and modify plant cell walls through the secretion of cellulolytic enzymes (ellulose, xylanase, pectinase, and endoglycanase) as a facilitator for the entry of bacteria to spread in plant tissues[43]. Endophytic bacteria generally originate from the rhizosphere because endophytic bacteria respond to plant root exudates[1], [41]. Colonization occurs starting from a process of reversible adsorption or irreversible adhesion mediated by extracellular proteins then synthesized by bacteria and controlled by chemical signals generated by the roots of the host plant[29], [30]. Plant exudates initiate microbial activity in the rhizosphere as a facilitator for attachment and entry of endophytic bacteria into plant roots[32], [44]. Certain endophytic bacteria initiate the colonization of tissues outside the roots such as stems and leaves throughout the plant endosphere[31], [32].

The mechanism of endophytic bacteria in increasing plant growth is through three metabolic pathways including phytostimulation, biofertilization, and biological control[30]. Phytostimulation is phytohormones that act as plant growth regulators such as modulation of phytohormonesauxin, gibberellins, cytokinins, and ethylene (ACC deaminase)[7], [45]. The bioavailability of nutrients has an important role in the biofertilization process[7]. In the process of biological nitrogen fixation, endophytic bacteria combine nitrogen in the atmosphere and convert it into ammonia, and transfer the molecule to plant metabolism[30]. Colonization of endophytic bacteria in plant tissue does not have a detrimental effect on other bacteria in the soil, so it is used as an alternative to maximize biological nitrogen fixation by plants[1].

IV.2 Stages of Identification of Endophytic Bacteria

Identification of endophytic bacteria is carried out to determine the classification of endophytic bacteria based on morphological characteristics, biochemistry, and physiological analysis[22], [46], [47]. In research[27]explained that identification was carried out by culturing the leaves and stems of the medicinal plant *Tinosporacordifolia* using LB (Luria Bertani) agar media with an incubation period of 48 hours at 37°C, this has similarities with several other similar studies[17], [47], [48]. 38 species of endophytic bacteria were obtained, consisting of 20 bacterial species from leaf tissue and 18 species from stem tissue[27]. In biochemical characterization, several species of bacteria were positive for catalase activity[6], [26], [47].

Molecular identification can be carried out after DNA extraction using the 16S rRNA gene which is amplified using specific primers for selected isolates[9], [25]. In addition, bacterial species can be performed using 16S rDNA gene sequencing or 16S rRNA gene sequencing with universal primers 27F and 1492R[9], [27]. The PCR assay can be carried out under the following conditions: an initial denaturation step at 95 °C for 6 minutes, followed by 35 denaturation cycles at 94 °C for 45 seconds, annealing at 57 °C for 1 minute, and an extension step at 72 °C for 1: 30 min, with the last extension step at 72 °C for 10 min[9]. The 16S rDNA or 16S rRNA genes from the selected isolates were sequenced and displayed in FASTA format, the results were sorted by the GenBank database sequence with the BLAST search system[9], [27], [47]. However, PCR products can also be sequenced by genome sequencing Dye Terminator Cycle Sequencing Genome LabTM according to the manufacturer's instructions[25]. Then for phylogenetic analysis, it can be done by aligning the strains of bacterial isolates obtained with bacterial strains that are in the GenBank database using the jPhydit program and MEGA software version 4.0, to build a phylogenetic tree using the Neighbor-Joining method using Kimura two-parameter[25], [39], [47]. Phylogenetic analysis of the isolates showed that the selected isolates were identified through PCR testing with 1200 bp fragment observations, and compared using BLASTN for sample sequences against the nucleotide database normally found in NCBI.[6], [10], [47].

Based on nucleotide identity and phylogenetic analysis, isolates from *Thymus Vulgaris* plants belong to 17 different genera and 30 bacterial species, with the most isolate species included in *Bacillus sp.*[9].In the phylogenetic analysis and gene sequencing of 23 isolates in 23 plants, the genus identified isolates, including isolates of endophytic bacteria in the roots of *Menthapulegium* (*Planomicrobium sp.*), isolates on roots of *Gundeliatournefortii* (*Bacillus sp.*), isolates on roots of *Achilleamillefolium* (*Staphylococcus sp.*) and isolates on stems of *Phaseolusvulgaris* (*Bacillus sp.*)[25]. In addition, in the identification of endophytic bacteria with antifungal activity based on 16S rRNA gene sequencing in various medicinal plants, two species of bacterial strains were confirmed, namely *B. pumilus* and *B. safensis*[3]. Then reported on research[27]38 isolates of endophytic bacteria consisting of 31 isolates included in *Bacillus sp.* 3 isolates including *Pseudomonas sp.* and 4 isolates including *Aneurinibacillussp.*In research [3]identification of endophytic bacterial isolates from 82 isolates, 33 isolates had antifungal activity.Theendophytic bacteria isolates were obtained from several medicinal plants, including*Thymus, Mint, German chamomile, Alhagi, Black nightshade, Yarrow, Mugworts, Tragacanth,* dan *Bitter almond*[3]. Isolates that had positive catalase activity were derived from medicinal plants*Mint, Yarrow, Tragacanth, Alhagi, Black nightshade,*dan*Mugworts*[3].

IV.3 Stages of Isolation of Endophytic Bacteria in Medicinal Plants

The stages of isolation of endophytic bacteria in medicinal plants include sample collection, surface sterilization, isolation of endophytic bacteria[26], [46]. Sampling is done by collecting several plant parts such as leaves, flowers, and fruits from different places[25], [26], [49], [50]. Sample sterilization was carried out by washing the sample using distilled water, dried, processed by a five-step surface sterilization procedure, including washing 4-10 minutes with 5% NaOCl, washing for 10 minutes with 2.5% Na2S2O3, washing 5 minutes with 75% ethanol, one washed with sterile water and finally rinsed with 10% Na2S2O3 for 10 minutes[10], [22], [50], [51]. Then all samples were dried at 100 °C for 10 minutes[25]. The root fragments were washed with running water to remove adhering soil and then shaken with an incubator shaker in water and tween 80 (1%) at 70 rpm for 10 minutes to reduce epiphytic microorganisms[10].

The sterility of the sample must be ensured through cultured tissue surfaces with blood agar media, incubated for 48 hours at 37 °C, then observed the colonies formed[25], [27], [47], [52]. Cultivation of endophytic bacteria was carried out with samples from each plant that had been aseptically crushed into small fragments, then cultured on International Streptomyces Project 2 (ISP2) agar, R3A agar, and blood agar, or with other media such as NA (Nutrient Agar), to the petiole and stem were placed upside down with the open part on the media plate while the leaves were placed on the NA media plate and covered with parafilm which was incubated for 2-4 days at 28 °C or 37 °C[25], [47], [52]. Endophytic bacterial isolates were identified based on their morphology and colony characteristics such as size, shape, color, margin, elevation, pigmentation, gram properties, and growth rate at different temperatures[49], [52]. Colonies with similar morphological characteristics are grouped in the same species[25]. Selected endophytic bacterial colonies were re-cultured with LB agar medium and all isolates were maintained at 4 °C for further testing[26], [27].

Endophytic bacteria produced from the plant *Calotropisprocera* is the highest number of endophytic bacteria isolated among other medicinal plants[26]. Based on the overall results, there were 129 endophytic bacterial isolates from Aloe vera roots, but only 32 isolates were selected for further studies based on their auxin production [10].

IV.4 Stages of Isolation of the Catalase Enzyme

Isolation of catalase enzyme from Bacillus sp. can be done after isolated and successfully identified[48]. In testing the catalase enzyme activity of bacterial colonies (aged 24 hours) mixed with a drop of 3% H_2O_2 on a slide, the appearance of bubbles indicates the production of the catalase enzyme[13], [22]. Another way is to measure the volume of potassium permanganate used during the titration of the decomposition of H_2O_2 into water and oxygen, or by the colorimetric method using dichromate/acetic acid reagents[12], [53]. In addition, the catalase enzyme activity test can be carried out using a spectrophotometric method which monitored the decrease in absorbance at maximum absorption of 240 nm caused by the decomposition ofH_2O_2[29], [47], [48], [54]. The reaction mixture consisted of 2.4 ml of 50 Mm phosphate buffer pH 7.0, added 0.5 ml of H_2O_2, and obtained 100 µl of extracellular crude extract.[29], [48]. One unit of catalase activity is defined as the amount of activity required to convert 1 µmol H_2O_2 into water and oxygen per minute at 25 °C[48]. The activity is expressed as mol H_2O_2 used every minute by taking the optical density at 240 nm[29]. Catalase activity can be calculated based on the H_2O_2 decomposition rate which is proportional to the reduction in H_2O_2 absorbance at λ maximum absorption of 240 nm[47], [55], [56].

Then the enzyme extraction was carried out, namely the isolated colonies were transferred into a 100 ml conical flask containing 50 mL of broth medium (8 peptones, 4 yeast extract, 2 NaCl with units of g^{-1} distilled water) with a pH of 7.2, incubation temperature of 3 °C with an incubator shaker at 150 rpm then centrifuged at 10,000 xg for 15 minutes at 4 °C[48], [56]. In addition, crude enzyme extracts can be prepared by ultrasonication method from fermented broth[56]. The clear supernatant which is an extracellular crude extract was used for enzyme assay[48]. Enzyme purification can be carried out by anion exchange chromatography[57]. The quantification of protein purification from the crude extract of enzymes was carried out by the Bradford method[56], [57].

The results of catalase enzyme activity can be reported as described by several studies as follows on Ginseng rhizosphere inoculated with TB6 (*B. amyloliquefaciens*) strain of 22.77%[12]. It was found that catalase activity increased in plants infected with *Pyriculariaoryzae* inoculated with endophytic bacteria *Pseudomonas pseudoalcaligenes* and *Pseudomonas aeruginosa*, which was 38.7 nmol/minute/g [29]. In the test of catalase activity, the bacterial isolate that had the highest extracellular catalase activity by *B. subtilis* was 135.2 M/ml among other *Bacillus* species with an estimated incubation time of 0-72 hours[48].s

IV.5 Stages of Catalase Enzyme Characterization

Characterization was carried out with purified crude enzyme including ammonium sulfate precipitation, anion exchange chromatography, and gel filtration[58], [59]. In the characterization of the hemecatalase

enzyme, several steps were carried out, including first determining the kinetic parameters (Km, Vmax, and Kcat), second determining the optimal temperature, pH, and thermostability conditions, third knowing the effect of H_2O_2 on cell growth and survival.[24], [60].

Crude enzyme extract prepared from *B. altitudinis SYBC hb4* was analyzed and separated by SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel)[29], [56], [59]. The visible bands were extracted and analyzed by MALDITOF/NOVA, the identified bands were compared with *B. stratosphericus*catalase (kat B), then the resulting fragments combined with the catalasestrain*B.altitudinis SYBC hb4*all had similarities with katB[59]. Kinetic parameters (Michaelis-Menten constant; Km, maximal reaction rate, Vmax, and turnover value, Kcat) by linear regression of the double-reciprocal plot according to Lineweaver and Burk[24], [56], [57], [59]. The resulting kinetic parameters can be shown in the table below:

Table no 2: Kinetic parameters of hemecatalase[24]**.**

Kinetic parameters	Catalase	Peroxidative
K_m	59,6± 1,3 mM	0,970 ± 2,5 mM
K_{cat}	322,651 x $10^3 s^{-1}$	19,42 s^{-1}
K_{cat}/K_m	5,41 x 10^3 $s^{-1}mM^{-1}$	20,02 $s^{-1}mM^{-1}$

Based on these results indicate a higher potential for hemecatalase to ensure faster H2O2 decomposition and can be used as a biocatalytic tool for food and biotechnology applications[24].

In determining the optimal temperature, pH, and thermostability conditions of pH, the optimal reaction was analyzed in 50 mM buffer (Sodium acetate (pH: 2-4), Sodium citrate (pH: 4-6), Potassium phosphate (pH: 6-8), Tris -HCl (pH: 8, 9), Glycine-NaOH (pH: 9-11) and Na2HPO4-NaOH (pH: 11-12))[24]. To measure stability and optimum pH, enzymes were incubated at 4 °C for 24 hours in different buffers[24], [60].The optimal temperature for the enzymatic activation of catalase was tested with different temperatures in the range (0 °C-90 °C) with an increase of 10 °C and temperature stability was tested by incubating the catalase enzyme at different temperatures (40 °C, 50 °C, 60 °C, 70 °C, and 80 °C) and different periods (15, 30, 60, and 120 minutes)[24], [56]. Hemecatalase showed good stability under 40 °C and 90% could maintain its activity after incubation at 20 °C for 20 hours, but the enzyme stability decreased significantly when it reached 60 °C in contrast to monofunctionalcatalase, hemecatalase had more peroxidase activity, and sensitive to inactivation by higher temperatures[24], [61].

The results obtained in the characterization of the catalase enzyme, one band shows pure catalase with a subunit molecular mass of about 57 kDa and a pure catalase molecular mass of about 228kDa with a Superdex 200 column[59].Based on these results, the purified catalase is a catalase tetramer consisting of four homo-subunits[59]. The catalytic activity of katB was determined based on the difference in pH and temperature values with maximum katB activity at a temperature of 30 °C and pH 5.0. The kinetic parameters of katB were analyzed by Lineweaver-Burk plot. The results obtained were Km 62 mM and Vmax 33.4 mol/min/mg, and B. altitudinis SYBC hb4 had potential as an acid-stable catalase[59].

IV.6 Application of Catalase Enzyme Produced by Endophytic Bacteria

In the medical field, the enzyme catalase is used as an inhibition of tumor metastasis and the treatment of catalasemia (catalase deficiency) associated with diabetes mellitus patients[48]. In the pharmaceutical field, catalase-peroxidase is used for the oxidation of -lactams to R-sulfoxide, in which B. pumilis bacteria act as biocatalysts and the enzyme responsible for oxidase is catalase-peroxidase (KatG)[56], [62], [63]. In the food industry, the enzyme catalase is used as a decomposer of peroxides during the pasteurization of milk[48]. Then catalase together with glucose oxidase can be used in food packaging with an active packaging method where the byproduct of active packaging is an oxidative species of H_2O_2 type so that it can protect food products from these oxidative species[8]. Hemecatalase can be used as a biocatalytic tool in the food industry and biotechnology applications by altering the antioxidant potential of aromatic compounds[24]. Then catalase can also be used as a therapeutic agent to fight various diseases related to oxidative stress such as acatalasemia, vitiligo, and neurological disorders (Alzheimer's and Parkinson's)[16]. In addition, in the textile industry, catalase is used to remove hydrogen peroxide from bleaching waste which has an environmental impact[8].

V. Conclusion

Colonization of endophytic bacteria in the host plant can be found on the stems, leaves, flowers, seeds, and mostly in the roots of medicinal plants. The method of isolation of endophytic bacteria used is cultivation.

Endophytic bacteria that have positive catalase activity are tested by dripping H_2O_2 reagent which produces gas/bubbles. Another method of testing the catalase enzyme is using the spectrophotometric method with a maximum absorption wavelength of 240 nm. While the identification of endophytic bacteria based on high accuracy is carried out by molecular identification with the 16S rRNA. Endophytic bacteria in medicinal plants are mostly identified as *Bacillus* species, some of which are *Bacillus pumilus*, *Bacillus cereus*, *Bacillus amyloliquefaciens*,and*Bacillussafensis*.

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