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Comparative Study of Rhodanese Specific Activity Assay Techniques for Plants and Bacterial Species

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Abstract

Rhodanese plays a critical role in cyanide detoxification by catalyzing the conversion of toxic cyanide into the less harmful thiocyanate. This comprehensive review examines recent studies on the purification and characterization of rhodanese from two distinct biological species such as plants and bacteria. In this study, different rhodanese assay techniques and purification methods are evaluated. The results showed that bacterial species, particularly *Bacillus cereus* and *Klebsiella oxytoca*, indicated the highest specific activity of 25.30 and 52.7 RU/mg, respectively, outperforming plant sources under similar purification conditions. Factors affecting enzyme activity such as temperature, pH, metal ion inhibition, and substrate specificity were also reviewed to minimize their effects on comparison by selecting nearly optimum temperature and pH, the highest affinity to the same substrate, and minimum effect of metal ion. The study concluded that bacterial species have greater rhodanese specific activity, and Lee modified by Agboola and Okonji and Bradford methods were the most used for rhodanese assay and protein quantification, respectively. Ion exchange chromatography is also the most effective rhodanese purification technique.

Keywords

Rhodanese, Specific Activity, Assay, Substrate Specification, Enzyme, Chromatography

1. Introduction

1.1. Background

Biochemical reactions within living organisms are catalyzed by enzymes. Enzymes play an essential role in various life processes, and their activity must be precisely

regulated to ensure proper cellular function [1] [2]. Enzymes are categorized into industrial enzymes, analytical enzymes, and medical enzymes based on their purposes, and they are highly specific catalysts with an active site in their structure where the substrate is converted into a product [3]. Furthermore, research has verified that a broad spectrum of enzymes has been isolated and identified from a wide array of biological origins [4]. Enzymes such as cyanide hydratase [5], 3-mercapto pyruvate sulfurtransferase [6] [7], cyanase [8], cyanide dihydratase [9], cyanide monooxygenase [10], and rhodanese [3]-[8] [11] have been identified as capable of detoxifying cyanide [12].

Rhodanese (thiosulfate: cyanide sulfur transferase, EC 2.8.1.1) is a universal enzyme found in all living organisms and catalyzes the conversion of cyanide into less toxic thiocyanate using a sulfur donor compound [13]-[15]. In a laboratory setting, it undergoes the irreversible transfer of a sulfur atom from thiosulfate to cyanide, resulting in the production of sulfite and thiocyanate [5]-[7] [16].

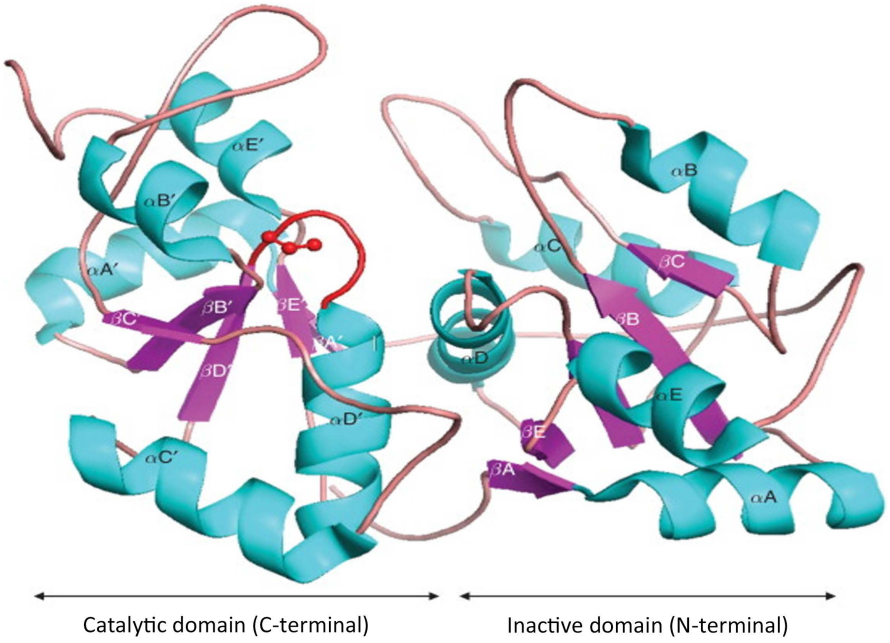
1.2. Conformational Structure of Rhodanese

Crystalline rhodanese, as isolated from a mammalian source, is a small monomeric sulfurtransferase with a molecular weight of ~32 kDa containing one reaction site per molecule. The bovine mitochondrial enzyme (Rhobov) is composed of a single polypeptide chain containing 293 amino acid residues [17]. Crystallographic analyses show the polypeptide chain is folded into two domains of equal size and similar three-dimensional tertiary structure [18]. The two structures are arranged around an approximately two-fold axis crossing the domain-domain interface Rhobov Fold as shown in **Figure 1**. Each domain is composed of a five-stranded parallel sheet, flanked on both sides by α-helices [19]. Hydrophobic interactions stabilize the inter-domain interface to form the active catalytic site. The catalytic residues are in the C-terminal domain, whereas the N-terminal domain stabilizes the active structure [20].

The inactive N-terminal and active C-terminal structurally show identical α/β topology in spite of low sequence identity [α-helix: cyan; β-sheets] [11] [18] [21]. **Figure 1** indicates the catalytic and non-catalytic structure of Rhodanese.

1.3. Mechanism of Action

The main process of eliminating cyanide from the body is facilitated by the rhodanese enzyme, which is found in liver mitochondria [15] [22] [23]. A close relationship has been found between rhodanese activity and cyanogenesis, suggesting that the enzymes play a role in enabling plants to convert cyanide through biotransformation processes [15] [24]. The active site of rhodanese is composed of six amino acid residues, with cysteine [Cys] at the first position, facilitating the catalytic process, and the Cys residue is localized in the N-terminal domain, where it is positioned adjacent to an aspartic acid [Asp] residue lacking catalytic function. The compound contains sulfhydryl and aromatic groups nearby, and its catalytic activity is mediated through a double displacement reaction, also known as



Source: [21].

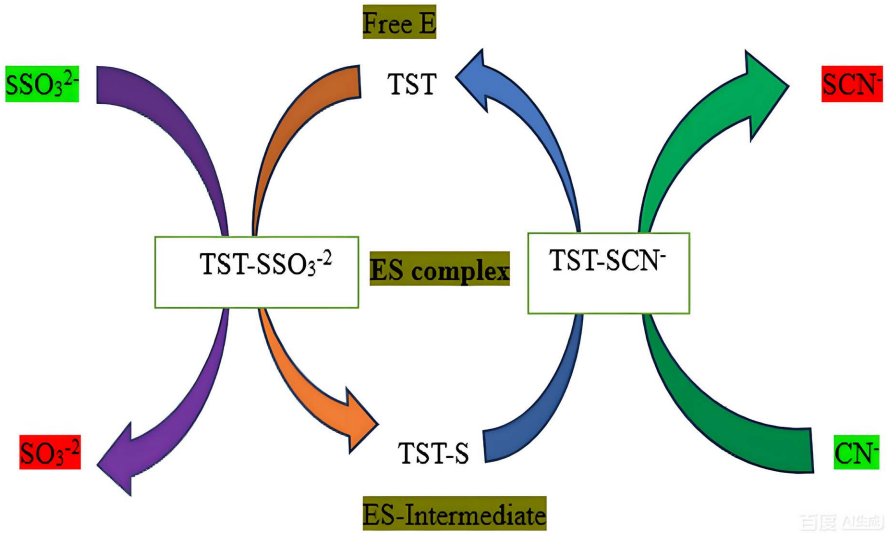
Figure 1. Conformational structure of mitochondrial rhodanese (Rhobov).

a ping-pong mechanism, where a stable persulphide-containing intermediate enzyme is formed [25] [26]. The process consists of two separate half-reactions. In the initial stage of the reaction, sulphur from the substrate is passed to the cysteine residue at the active site, where it forms a persulphide enzyme intermediate [27]. In the latter part of the reaction sequence, a thiophilic acceptor binds to the enzyme-bound persulphide intermediate, giving rise to the product and restoring the free enzyme [15] [27]. The sulfur being transferred is attached to a catalytic cysteine residue that remains constant. The thiocyanate is formed through a nucleophilic reaction with cyanide, formally denoted as S^0, which results in the regeneration of the active Cys residue for an additional cycle of the reaction. The catalytic mechanism of rhodanese involves transferring thiosulfate to a nucleophilic acceptor [28] [29]. **Figure 2** illustrates the stepwise double displacement (ping-pong) catalytic mechanism of rhodanese, showing how the enzyme transfers sulfur between substrates through a covalent enzyme-sulfur intermediate.

The general reaction is

$$\underset{\text{Thiosulphate ion}}{S_2O_3^{2-}} + \underset{\text{Cyanide ion}}{CN^-} \xrightarrow{\text{rhodanese}} \underset{\text{Thiocyanide ion}}{SCN^-} + \underset{\text{Sulphite ion}}{SO_3^{2-}} \quad (1)$$

In animal systems, roughly 65% - 80% of the cyanide dosage is converted into thiocyanate via a sulfur donor and free cyanide [30] [31]. Significantly increasing the rhodanese dosage results in a substantial enhancement of the HCN degradation efficiency rate [14]. Rhodanese has been extracted, purified, and characterized from several classes of species due to its cyanide detoxification function [19].



TST = Thiosulphate Sulphur Transferase (Rhodanese).

Figure 2. Stepwise double-displacement catalytic mechanism of rhodanese.

1.4. Biological Source of Rhodanese

Rhodanese was first identified by Lang in 1933 and is found in a broad range of organisms, including both prokaryotes and eukaryotes [15] [32] [33]. It exists in all living organisms, ranging from bacteria to humans. The organism's function has been assessed in bacteria, yeast, plants, and animals, but how it is distributed across various tissues seems unique to each species [19]. The organism has been found in various animal tissues such as the liver, proventriculus, esophagus, gizzard, cecum, brain, kidney, and lungs, where it is involved in the mitochondrial chain. The liver is the primary source of rhodanese, with the kidney being a secondary source, and rhodanese is also highly prevalent and extracted from a variety of cyanogenic and non-cyanogenic plants [15]. Rhodanese activity in higher plants is significantly lower than in animals, and significantly higher in cyanogenic plants compared to non-cyanogenic plants [34].

This review paper assesses the specific activity of recently published research on purification of rhodanese from bacteria and plants. The main goal of assessing rhodanese activity is to identify the most common assaying and quantifying techniques used in the rhodanese enzyme extraction, and its presence in samples under specific conditions, thereby enabling the comparison of activity levels across different samples and measurement methodologies. The detailed procedure is outlined in the Rhodanese specific activity determination section.

2. Rhodanese Specific Activity Determination Techniques

Rhodanese enzyme activity has been documented in multiple plant species, including cassava leaves, *Pentadiplandra brazzeana* root, almond, snake tomatoes, meal tomatoes, locust bean seed, maize, and corn. In bacteria like *Bacillus cereus*, Bacillus licheniformis, wild *Klebsiella oxytoca*, and Klebsiella edwardsi, the activity of rhodanese is examined. The total rhodanese assay and total protein concen-

tration need to be established before calculating the specific activity.

2.1. Methods for the Rhodanese Assay

Several methods for rhodanese assay have been described. All the rhodanese assay methods, including the Agboola and Okonji method, the modified Lee method, the classical Sorbo method, the Sorbo-Aminlari method, and the Modified Sorbo method, are fundamentally based on the same colorimetric principle: enzymatic conversion of cyanide to thiocyanate, followed by formation of a ferric-thiocyanate complex measured at 460 nm [35]. However, these methods differ in practical parameters such as reaction volume, enzyme volume, substrate concentrations, incubation time, and temperature. Despite these procedural variations, all techniques operate as minor modifications of the same underlying thiocyanate-based colorimetric assay for quantifying rhodanese activity.

The majority of the rhodanese assay methods utilized the approach developed by Agboola and Okonji. The rhodanese assay method measures enzyme activity, which is typically quantified in Rhodanese Units (RU). According to reference [28], the amount of enzyme that yields an optical density measurement of 1.08 at a wavelength of 460 nm is the unit of one RU. The amount of rhodanese that catalyzes the conversion of one micromole (1 μmol) of cyanide ion to thiocyanate in a minute is defined as a rhodanese unit [14] [36]. The details of these methods are explained as follows.

The Lee method, as modified by Agboola and Okonji, is articulated through the utilization of a reaction mixture comprising 0.5 ml of 50 mM borate buffer (pH = 9.4), 0.2 ml of 250 mM potassium cyanide (KCN), and 0.2 ml of 250 mM sodium thiosulfate ($Na_2S_2O_3$), along with 20 μl of enzyme solution, culminating in a total volume of 1.0 ml; this mixture is subsequently incubated for 1 minute at a temperature of 37 °C, after which the reaction is terminated by the introduction of 0.5 ml of 15% formaldehyde, followed by the incorporation of 1.5 ml of Sorbo reagent. Ultimately, the absorbance is measured spectrophotometrically at a wavelength of 460 nm [14] [37] [38].

The methodology proposed by Agboola and Okonji is characterized by a reaction mixture comprising 250 mM $Na_2S_2O_3$, 250 mM KCN, and 50 mM borate buffer at pH 9.4, alongside 100 μl of enzyme solution within a final volume of 1.0 ml. 1.5 ml of Sorbo reagent and 0.5 ml of 15% formaldehyde are added to stop the reaction after it has been running for 1 minute at 37˚C. A ferric nitrate solution containing 0.025 g $Fe(NO_3)_3 \cdot 9H_2O$, diluted in 0.74 ml of water and 0.26 ml of concentrated nitric acid, is combined to create the Sorbo reagent. Finally, a wavelength of 460 nm is used to measure the absorbance [25] [35] [36] [38]-[40].

The Sorbo method is explained with an assay mixture of 50 mM borate buffer, pH 9.4, 0.25M KCN, 0.25M $Na_2S_2O_3$, and 0.1 ml of the enzyme solution in a total volume of 1 ml. After 1 minute of incubation at 37˚C, the reaction is terminated by adding 0.5 ml of 15% formaldehyde. The concentration of thiocyanate produced is determined by the addition of 1.5 ml of Sorbo reagent, where the Sorbo

reagent is made up of 10 g $Fe(NO_3)_2 \cdot 9H_2O$, 20 ml HNO_3, and 80 ml distilled water. Then, the absorbance of the reaction medium is read at 460 nm [37].

0.25 ml of 50 mM borate buffer, pH 9.4, 0.1 ml of 250 mM KCN, 0.1 ml of 250 mM Na_2S_2O, and 0.1 ml of the enzyme solution make up the reaction mixture used in the Sorbo and Aminlari technique, which has a total volume of 0.55 ml. After one minute of incubation at 37˚C, 0.25 mL of 15% formaldehyde is added to the reaction mixture to halt it. By adding 2.5 mL of 1% ferric nitrate to 13% nitric acid reagent, the spectrophotometric generation of thiocyanate is measured, and the optical density is measured at 460 nm [37] [41]. A 25 mM citrate-phosphate-borate buffer at a pH of 9.4, 50 mM potassium cyanide (KCN), 50 mM sodium thiosulphate ($Na_2S_2O_3$), and 50 µl of enzyme solution make up the reaction mixture used in the modified Sorbo technique, which has a total volume of 1.75 ml. After incubating the mixture for two minutes at 25˚C, 0.25 mL of 37% formaldehyde and 1 mL of Sorbo reagent are added to stop the reaction. At 460 nm, the absorbance is measured [41].

The modified Lee method is elaborated as follows: the assay mixture contains 50 mM borate buffer, pH 9.4, 200 mM $Na_2S_2O_3$, 200 mM KCN, and 200 µl of enzyme in a total volume of 1 ml. The mixture is incubated with 0.5 ml of 15% formaldehyde for 1 min at 37˚C, followed by the addition of an appropriate volume of Sorbo reagent. The absorbance is then read at 460 nm using a spectrophotometer [25].

2.2. Rhodanese Purification Methods

Many enzyme purification methods have been developed over the years. The purification is accomplished in two different stages, *i.e.*, ammonium sulfate precipitation and column chromatography separation [42]. Column chromatography separation includes affinity chromatography on reactive Blue-2 Agarose [43]-[45], ion exchange chromatography on CM-Sephadex C-50 [46] [47], Sephadex G-150 gel filtration chromatography [48] [49], and Sephadex G-75 gel filtration. Traditional enzyme purification procedures often start with ammonium sulfate precipitation [50]. Chromatographic separation of protein mixtures has become one of the most effective and widely used means of purifying individual proteins [51].

Several rhodanese purification techniques have also been observed for various biological extracts. 65% - 80% ammonium sulphate precipitation [52], affinity chromatography on reactive Blue-2 Agarose, ion exchange chromatography on CM-Sephadex C-50 & 25, Sephadex G-150 gel filtration chromatography, and Sephadex G-75 gel filtration are the main rhodanese purification techniques observed during the review.

2.3. Rhodanese Quantification Methods

Determining the concentration of protein is critical in numerous contexts, encompassing enzyme kinetics, enzyme purification, and enzymatic testing. Three of the most widely used methods for protein quantification on a large scale are the Lowry, Bicinchoninic, and Bradford methods [53]-[57]. One of the most commonly used methods for protein purification is the Bradford assay. This method

utilizes the binding of Coomassie Brilliant Blue dye to basic amino acid residues in the protein, resulting in a shift in the dye's absorbance spectrum. The amount of protein present in the sample can then be quantified by measuring the absorbance of the dye-protein complex at 595 nm [57]. This method is utilized to measure the concentration of enzymes after purification, employing bovine serum albumin (BSA) as a standard [28] [58]. Bovine serum albumin [BSA] is the most commonly used standard in protein concentration quantification [59]. For the Bradford enzyme quantification assay to work, basic amino acid residues such as arginine, histidine, phenylalanine, tryptophan, and tyrosine must bind to Coomassie Brilliant Blue G-250 dye at an acidic pH. Hydrophobic interactions in proteins also cause this dye to change colour to blue [39] [54].

The second method applied in the quantification of rhodanese is the Lowry method [60]. This method involves the reduction of copper ions in the presence of protein, resulting in the formation of a complex between the proteins and reduced copper ions. The formation of this complex can be detected by measuring the absorbance of the resulting solution at 750 nm [57]. The Bicinchoninic Acid (BCA) assay is another widely used method for protein quantification. This assay is based on the reduction of Cu^{2+} ions by protein in an alkaline environment to form a complex with BCA, resulting in a color change that can be detected spectrophotometrically at 562 nm [53] [57] [61]. The BCA assay proves to be more specific than the Bradford and Lowry assays, while also displaying reduced sensitivity to interfering substances in the sample [54] [61] [62].

2.4. Calculation of Specific Activity

Enzyme activity assays determine the rate at which an enzyme speeds up a chemical reaction, with that rate directly linked to the amount of enzyme present in the sample. Direct measurement methods can sometimes be less accurate than enzyme activity assays, which consider the actual activity of the enzyme [63]. The specific activity of an enzyme is directly related to its purity within a protein composition. It measures the activity of a specific enzyme on a per-milligram basis of the total protein present. This process is notably important in isolating enzymes from particular proteins to assess their purity. The specific activity is calculated by dividing the number of enzyme units per milliliter by the concentration of protein in milligrams. In the case of Rhodanese, this value is expressed in units of RU per milligram [3] [64].

$$\text{Rhodanese specific activity}\left(\frac{\text{RU}}{\text{mg}}\right)=\frac{\text{Rhodanese enzyme activity, RU/ml}}{\text{Total protein concentration, mg/ml}} \quad (1)$$

2.5. Factors Affecting Enzyme Activity

A range of factors can impact enzyme activity, including temperature [65]-[68], pH levels [67]-[70], the presence of metal ions [71] [72], and substrate concentration levels [73]-[75]. The optimum temperature of rhodanese has been reported from 45˚C - 60˚C, while optimum pH values range between 6 and 8.0 for different sources. Another key consideration is the enzyme's selectivity for its substrate.

The enzyme's substrate specificity was examined by assessing its activity towards structurally analogous compounds. The effect of various substrates [sodium thiosulphate, sodium metabisulphite, and ammonium persulphate] has been investigated by calculating the Michaelis-Menten constants [Vmax and Km] [37]. Research has documented the substrate specificity of rhodanese isolated from plants, bacteria, and animals. The impact of these heavy metal compounds [$BaCl_2$, $NiCl_2$, KCl, NaCl, $ZnCl_2$, $CaCl_2$, $HgCl_2$, $SnCl_2$, and $MnCl_2$] at concentrations of 1 and 10 millimolar in the reaction mixture on rhodanese activity is documented [76]. Although the cited studies investigated rhodanese activity across a range of temperature, pH values, substrate concentrations, and metal ion levels, this study focused only on an optimum operating condition, *i.e.*, 50˚C and a pH of 8.0, as well as its strong affinity for sodium thiosulfate and minimal interference from metal ions. Studying these factors is crucial for comparing the enzyme activities obtained from recently isolated rhodanese. **Table 1** represents rhodaneses' assaying methods, purification techniques, and specific activity for plants and bacterial species.

Table 1. Rhodanese-specific activity and its reliance on biological sources.

Biological Sources	Enzyme assay procedures/protein quantification methods	Protein purification methods	Results			Reference
			Total activity, RU	Total protein, mg	Specific activity RU/mg	
Cassava leaves	Lee modified by Agboola and Okonji/Bradford	80% Ammonium Sulphate Precipitation	145.5	965.3	2.03	[14]
Bitter cassava	Lee modified by Agboola and Okonji/Bradford	80% Ammonium Sulphate Precipitation	791.32	263.51	3.00	[38]
		Ion-exchange Chromatography on CM-Sephadex C-25	338.51	63.22	5.35	
Almond (*Prunus amygdalus*) nuts	Agboola and Okonji/Bradford	80% Ammonium Sulphate Precipitation	85.77	29.7	2.89	[25]
		Ion-exchange chromatography on CM-Sephadex C-50	54.24	11.32	4.79	
		Reactive Blue-2 Agarose Affinity Chromatography	26.79	5.26	5.09	
Seed and mesocarp of snake tomatoes (*Trichosanthes cucumerina* Linn)	Agboola and Okonji/Bradford	80% Ammonium Sulfate Precipitation	S: 41.3 M:81.7	S: 2.1 M: 9.3	S: 19.7 M: 8.8	[77]
Tomato (*Solanum lycopersicum* Mill)	Agboola and Okonji/Bradford	80% Ammonium Sulphate Precipitation	150.63	53.9	2.79	[78]
		Ion-exchange Chromatography on CM-Sephadex C-50	94.54	31.45	3.01	
		Biogel P-100 Size Exclusion Chromatography	56.1	12.62	4.45	

Continued

Seed, Mesocarp, and Capsule of Snake Tomato Fruit	Lee, modified by Agboola and Okonji/Bradford	80% Ammonium Sulphate Precipitation	S:74.39 M:5.5 C: 6.56	S:1480.86 M:332.65 C:737.87	S: 0.0502 M: 0.0165 C:0.0089	[79]
		Ion-exchange chromatography on CM-Sephadex C-25	S:0.32	S: 10.16	S: 0.0315	
		Reactive Blue-2 Agarose Affinity Chromatography	S:7.604	S:0.742	S:0.0621	
Locust bean seed (*Parkia biglobosa*)	Sorbo and Aminlari /Bradford	80% Ammonium Sulfate Precipitation	105.77	33.7	3.14	[80]
		Ion-exchange Chromatography on CM-Sephadex C-50	74.24	20.11	3.69	
		Reactive Blue - Affinity Chromatography	36.79	7.06	5.21	
Pentadiplandra brazzeana (Baill) root	Agboola and Okonji/Bradford	80% ammonium sulphate precipitation	71.80	31.12	2.31	[81]
		Reactive Blue - Affinity Chromatography	15.6	3.24	4.82	
Malted maize (*Zea mays*)	Lee, modified by Agboola and Okonji/Bradford	85% Ammonium Sulphate Precipitation	102.87	21.06	4.88	[82]
		Ion-exchange Chromatography on CM-Sephadex C-25	9.88	1.95	5.07	
Seed of Guinea Corn (*Sorghum arundinaceum* Stapf)	Lee, modified by Agboola and Okonji/Bradford	Acetone Precipitation	43.09	5924.29	0.007	[83]
		Biogel P-100 Gel Filtration	1.59	57.88	0.027	
		DEAE–Sephadex ion-exchange chromatography	0.18	7.00	0.0026	
Bacillus cereus	Sorbo/Bradford Sorbo	80% Ammonium Sulphate Precipitation	52.095	6.053	8.74	[37]
		Ion-exchange Chromatography on CM-Sephadex C-50	52.40	2.27	23.08	
		Biogel P-100 Size Exclusion Chromatography	33.88	1.339	25.30	
Bacillus licheniformis	Lee/Bradford	85% Ammonium Sulfate Precipitation	59.08	20.25	2.92	[40]
		Ion-exchange chromatography on CM-Sephadex C-50	4.77	0.39	12.23	
		Sephadex G-100 gel filtration chromatography	20.42	1.50	13.63	
Klebsiella edwardsii	Modified Sorbo/Bradford	85% Ammonium Sulphate Precipitation	4.80	205.625	0.0234	[41]
		Ion exchange on DEAE Cellulose	8.07	170.625	0.0473	

Continued

Wild Klebsiella Oxytoca	Agboola and Okonji/Bradford	70% Ammonium Sulfate Precipitation	239.7	6.2	38.6	[39] [84]
		Sephadex G-100 gel filtration chromatography	336.13	97.02	3.47	
		Ion-exchange chromatography on CM-Sephadex C-25	213.5	4.1	52.7	

3. Discussion

The evaluation and comparison of rhodanese distribution in plants and bacteria, and the effectiveness of different purification techniques, were assessed in this review. Studies from diverse plant and bacterial species consistently demonstrate substantial variation in rhodanese specific activity, reflecting the strong influence of biological source and purification methodology.

In nearly all studies, ammonium sulfate precipitation from 70% - 85% concentration served as the initial crude purification step. This produced moderate specific activities in plant sources such as cassava (2.02 RU/mg) and almond (2.89 RU/mg), but extremely low activity in guinea corn seeds (0.007 RU/mg). In contrast, *Bacillus cereus* and *Bacillus licheniformis* exhibited considerably higher values of 8.74 RU/mg and 2.92 RU/mg, respectively, after the initial precipitation procedure. Further purification through ion-exchange chromatography markedly enhanced activity to 23.08 RU/mg in *Bacillus cereus* and 12.23 RU/mg in Bacillus licheniformis. The highest activity reported across all studies was found in wild *Klebsiella oxytoca*, reaching 52.7 RU/mg after ion-exchange purification.

Plant materials such as tomato and snake tomato generally showed only modest improvements after purification in chromatography. However, Reactive Blue-2 agarose affinity chromatography demonstrated strong potential for plant extracts, yielding specific activities of 5.05 RU/mg in almond, 5.21 RU/mg in locust bean, and 4.82 RU/mg in *Pentadiplandra brazzeana* root. This suggests that affinity chromatography may be particularly suitable for enhancing plant rhodanese purity.

Conversely, size-exclusion chromatography techniques, Biogel P-100 and Sephadex G-100, were more effective for bacterial sources, producing activities of 25.3 RU/mg and 13.63 RU/mg in Bacillus licheniformis. Despite undergoing multiple purification steps, Klebsiella edwardii consistently displayed low specific activities of 0.0234 - 0.0473 RU/mg, indicating limited intrinsic enzyme abundance or stability in that species.

In comparing the rhodanese activity assay techniques, most studies relied on the Abgoola and Okonji and modified Lee methods, while others applied variations of the classical Sorbo assay. Although all are based on the same thiocyanate-ferric complex colorimetric principle, differences in reaction volumes, incubation times, enzyme quantities, and substrate concentrations can yield slight variation in measured activity. Likewise, for protein quantification, the Bradford assay

dominated due to its dye-protein interaction and minimal interference, while fewer studies employed the Lowry and BCA methods. Such differences in assay and quantification techniques may introduce small variations in calculated specific activity.

Although the cited studies evaluated rhodanese activity under a broad range of temperatures, pH values, substrate concentrations, and metal-ion levels, the comparison presented in this review relies extensively on the optimum conditions and minimal-inhibitor states reported in each study. By focusing only on the peak activity parameters, major variability among methods was minimized. Nonetheless, minor differences in extraction procedures, buffer compositions, and assay setups may still contribute to small variations in reported activity. These methodological differences represent a minor limitation when making direct cross-study comparisons.

The consistently observed rhodanese activities in bacteria may arise from several biological and biochemical factors. Bacterial systems often exhibit higher enzyme expression levels as part of their adaptive response to harsh or cyanide-rich environments. In addition, bacterial rhodanese may possess inherently greater catalytic efficiency and superior folding stability compared to plant enzymes (36). The simpler cellular matrix of bacteria, lacking complex polysaccharides, secondary metabolites, and phenolic compounds common in plant tissues, also facilitates more efficient extraction and purification, reducing potential interference that can depress measured activity.

4. Conclusion

This review highlights the substantial differences in rhodanese specific activity among various biological sources and purification techniques. Across all examined studies, bacterial species consistently demonstrated significantly higher specific activity compared to plant sources, particularly when purified by ion exchange and gel-filtration chromatography. The practical implication of this trend is that bacterial species, especially *Bacillus cereus*, *Bacillus licheniformis*, and wild *Klebsiella oxytoca*, represent promising candidates for industrial and environmental applications. Overall, the evaluated purification and assay approaches confirm that bacteria provide a more efficient and scalable source of rhodanese for future industrial applications. The Agboola and Okonji and modified Lee assay techniques emerged as the most widely applied methods for determining rhodanese activity, while the Bradford assay remained the dominant approach for protein quantification.

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Availability of data and materials

The datasets used are available from the corresponding author upon reasonable request.

Ethical statement

This article is a review and does not involve any new experiments on humans or animals. All data discussed are taken from previously published studies and are properly cited.

Author's Contribution

Badhane G. Collect recently published data that are relevant to the title, and write the manuscript, recheck, and evaluate by comparing with various published papers.

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this review paper.

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Abbreviation

BCA	Bicinchoninic Acid
BSA	Bovine Serum Albumin
CM	Carboxymethyl
CM-Sephadex	Carboxymethyl Sephadex
DEAE	Diethylaminoethyl
$Fe(NO_3)_3{\cdot}9H_2O$	Ferric Nitrate Nonahydrate
G-100/G-150	Gel Filtration Chromatography Media (Sephadex)
HNO_3	Nitric acid
KCN	Potassium cyanide
K_m	Michaelis constant
RU	Rhodanese unit
SO_3^{2-}	Sulphite ion
V_{max}	Maximum velocity (in enzyme kinetics)