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ISOLATION AND CHARACTERIZATION OF ANTIMICROBIAL BACTERIAL ENDOPHYTES AGAINST FOODBORNE PATHOGENS FROM COFFEA ARABICA & COFFEA ROBUSTA FROM CHIKMAGALUR

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Abstract

Endophytes are microorganisms that reside within plant tissues without causing any harm to the host. These non-pathogenic symbionts are known to produce various bioactive compounds, including antibiotics. Recent studies have emphasized the potential of endophytic bacteria in addressing the escalating threat of antibiotic resistance among pathogenic microorganisms. Chikmagalur, widely recognized as the birthplace of Indian coffee and known as the "Coffee Land of Karnataka," is renowned for the cultivation of *Coffea arabica* and *Coffea robusta*. In the present study sixteen bacterial endophytic isolates were obtained from different parts including root, stem, leaves and bark of *Coffea arabica* & *Coffea robusta* from Chikmagalur. The antimicrobial screening of the ethyl acetate extract of the isolates against foodborne pathogens, such as *Salmonella typhi*, Shigella, *E. coli* & *Enterococcus faecalis* was done using agar diffusion method. Eight out of sixteen endophytic bacterial isolates exhibited antimicrobial activity against the tested foodborne pathogens. Among them, two isolates CCRBI and CCRB2, both obtained from the bark of *Coffea robusta* demonstrated notably higher antimicrobial activity, showing larger zones of inhibition against *Salmonella typhi*, *Shigella* spp., and *Escherichia coli*. Consequently, these two potent isolates were selected for detailed morphological, biochemical, and phytochemical characterization.

Bioactive compounds were extracted from the liquid cultures of CCRBI and CCRB2 using ethyl acetate, and the resulting crude extracts were subjected to phytochemical analysis to identify the secondary metabolites. The ethyl acetate extract of CCRBI exhibited inhibition zones of 20.5 mm against *S. typhi*, 26.5 mm against *Shigella*, and 24 mm against *E. coli*. Similarly, CCRB2 showed inhibition zones of 20.5 mm, 21.5 mm, and 27 mm against the same pathogens, respectively. Phytochemical screening revealed the presence of alkaloids and saponins in the crude extracts, indicating their potential role in the observed antimicrobial activity.

Keywords: Antibacterial, endophytic bacteria, Chikmagalur, *Salmonella typhi*, *Shigella dysenteriae*, *E. coli*, *Enterococcus faecalis*, Phytochemical

1. INTRODUCTION

The emergence and spread of antimicrobial resistant pathogens are increasing progressively making the currently available antimicrobial drugs less effective (Uche et. al., 2019). In recent years, there has been a growing interest in the study of medicinal plants and their endophytes as alternative sources for bioactive compounds against resistant pathogens. Endophytes are plant origin microbials which cause no harm to the host, they are non-pathogenic symbionts and their adopt varies. They are found in every plant on the earth. Soil and geographical conditions contribute to the types of endophytes which colonize the plants.

Traditionally, the quality and quantity of crude drugs from medicinal plants were attributed to plant genetics, habitat and soil nutrients. However, recent studies recognize that endophyte–host interactions also play a crucial role in influencing these properties (Jia et al., 2016)

The endophytes produce a plethora of potential substances which can be used in medicine. Novel antibiotics,

immunosuppressants, and anticancer compounds were isolated, purified and characterized from notable endophytes in recent past. Therefore, the potential likelihood of finding new drugs for newly developed diseases in humans, plants and animals are significant (Strobel & Daisy, 2003).

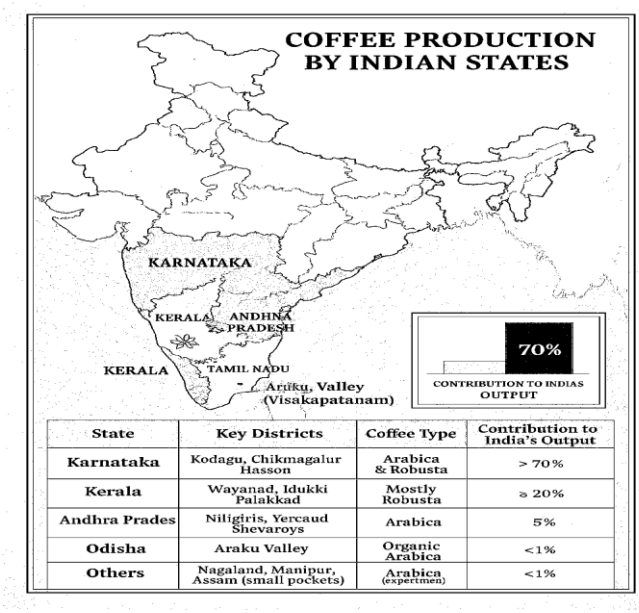
Nearly 50% of clinically used drugs are natural compounds or their derivatives, largely due to the pharmacological activities of phytochemicals such as alkaloids, terpenoids, flavonoids, lignans, and glycosides (Kumar & Mathur, 2017). Extensive research has revealed a wide array of chemicals from endophytic microbes, which are important sources for drug discovery (Gouda et al., 2016). Moreover, bioactive compounds from endophytes remain largely unexplored, and untouched microbial habitats may provide additional sources of useful metabolites (Qin et al., 2011). Over the last decade, endophytes have gained attention as sources of bioactive compounds, since many endophytes associated with medicinal plants produce the same metabolites as their hosts, therefore medicinal plants are explored to isolate endophytes capable of yielding novel antimicrobial substances of pharmaceutical value (Tanvir et al., 2017). The microbes are known as producers of growth promoting metabolites, insect and pest repellents, antimicrobials against plant pathogens, and compounds that enhance stress tolerance, amongst other functions (Rai et al., 2014a, b). They also have the ability to synthesize unique secondary metabolites with applications in pharmaceutical, agricultural, and industrial sectors. Consequently, there is increasing research interest in bioprospecting endophytic microbial communities inhabiting plants from diverse ecosystems (Vasundhara et al., 2016). Endophytes are a reservoir of novel bioactive secondary metabolites like alkaloids, phenolic acids, quinones, steroids, saponins, tannins and terpenoids which are antimicrobial, anti-cancerous and also potential antioxidants. (Gouda et. al., 2016).

Coffee plants are evergreen shrubs belonging to *Rubiacea* family, which consists of 450 genera and 6500 species. The most famous species of the Coffea Genera being, *C. arabica*, *C. robusta*, and *C. liberica*. *Coffea* species are widely distributed across the world and hold significant scientific importance due to their pharmacological properties. Coffee consumption has been growing globally due to its pleasant aroma and health benefits. Various studies have revealed the health benefits of coffee as a diuretic, as an antimicrobial and due to its antioxidant activities. (Mahajan & Kapoor, 2018).

Owing to their rich caffeine and polyphenol content, *Coffea* extracts exhibit diverse physiological benefits, including effects on the central nervous system, along with antioxidant, anticancer, gastrointestinal, cardiovascular, antibacterial, and dermatological activities. Several scientific studies have reported the beneficial effects of coffee on human health. Its phytochemicals—primarily chlorogenic acids, caffeine, diterpenes, and trigonelline are considered responsible for these effects.

According to the Food and Agriculture Organization, coffee is the fourth most valuable agricultural traded commodity and the most consumed beverage globally (Munyendo et.al., 2021).

In India, coffee is cultivated mainly in the Southern States of India: Karnataka – 54% Kerala – 19% Tamil Nadu – 8% figure 1.



State	Key Districts	Coffee Type	Contribution to India's Output
Karnataka	Kodagu, Chikmagalur Hasson	Arabica & Robusta	> 70%
Kerala	Wayanad, Idukki Palakkad	Mostly Robusta	≈ 20%
Andhra Prades	Niligiris, Yercaud Shevaroys	Arabica	5%
Odisha	Araku Valley	Organic Arabica	<1%
Others	Nagaland, Manipur, Assam (small pockets)	Arabica (expertmen)	<1%

Figure 1: Coffee growing states of India

Karnataka being the largest producer of Coffee, the districts Chikmagalur, Kodagu and Hassan are the main Coffee

Districts of Karnataka.

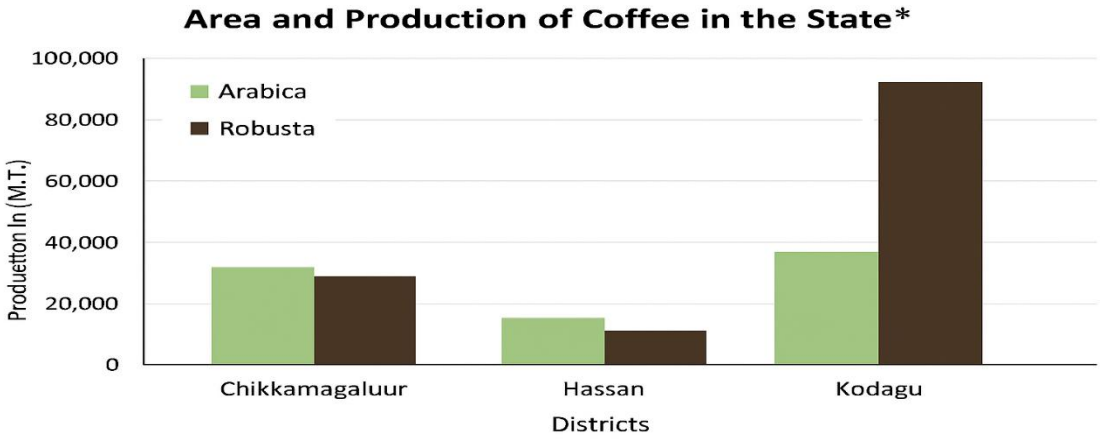


Figure 2: Area & Production of Coffee in Karnataka

The history of coffee in Karnataka originated from Chikmagalur a district of Karnataka. Chikmagalur is known as the "land of coffee". The origin of coffee cultivation in India is attributed to the Sufi saint Baba Budan, who introduced coffee seeds from Arabia and planted them in his garden at Chikmagalur. The potentiality of coffee endophytes in plant growth promotion through enhanced nitrogen fixation, availability of minerals, nutrient absorption, secretion of phytohormones, and other bioactive metabolites has been well documented (Asad et. al., 2023). Screening endophytes for antimicrobial compounds is considered a promising approach to encounter the growing threat of drug-resistant human and plant pathogens (Yu et al., 2010)

Bioprospecting of potential microbes for drug discovery involves their isolation, structural elucidation of metabolites, and investigation of biosynthetic pathways. Endophytes capable of producing the same bioactive compounds as their host plants not only reduce the need to harvest slow-growing or rare species but also help preserve global biodiversity. Moreover, microbial production of valuable compounds is often easier and more cost-effective, thereby reducing market prices. In this study, bacterial endophytes from *Coffea arabica* and *Coffea robusta* collected from Chikmagalur, Karnataka, were isolated and characterized. The isolates were screened for antimicrobial activity against major foodborne pathogens including *Salmonella typhi*, *Shigella* spp., *Escherichia coli*, and *Enterococcus faecalis*. Crude and ethyl acetate extracts from liquid cultures of the isolates were tested using the agar diffusion method, revealing their potent inhibitory activity. Promising isolates underwent detailed morphological, biochemical, and phytochemical characterization to identify active secondary metabolites. The findings underscore the potential of coffee-associated endophytes as novel sources of antimicrobial compounds in the fight against multidrug resistance.

2. METHODOLOGY:

2.1. Sample Collection:

The common species of Coffee plants, *Coffea arabica* and *Coffea robusta* were obtained from Chikmagalur, Karnataka. All the parts of the Coffee plants like the roots, the stem, the leaves, the beans and the flowers were used for the isolation of the endophytes. These parts of the plant were collected in separate zip-lock covers respectively labelled and transported to Bangalore.

2.2. Sample preparation & Surface sterilization of the plant parts:

The plant parts were stored in a refrigerator for not more than 2 days before use for the isolation of endophytes.

The first and obligatory step for endophyte isolation is to kill all the surface microbes. It was accomplished by treatment of plant parts with an oxidant or a general sterilant for a period of time, followed by a rinse or a wash with sterile distilled water. 70– 95% ethanol and 3–10% sodium hypochlorite was used. (Qin, *et, al.,* 2011)

2.3. Isolation of endophytes from the plant parts:

For the isolation of endophytic bacterial isolates, the stem, the leaves, roots, flowers, bark and the beans of healthy coffee plants (*C. arabica* & *C. robusta*) were used. The plant parts were washed thoroughly under running tap water and surface sterilized (70 % C_2H_5OH, 3 min, 0.5 % NaOCl, 5 min and 70 % C_2H_5OH, 1min). The sodium hypochlorite treatment duration was altered from 3 minutes to 5 minutes to optimize the surface sterilization process. Finally, they were washed six times with sterile distilled water. Surface sterilization efficiency of the sterilizers was checked by

inoculating the sixth wash water on nutrient agar plate, labelled as control. The surface sterilized plant parts were blot dried, sliced into thin sections and placed aseptically over nutrient agar plate petri dishes and incubated at 37 °C for 2-4 days in a bacteriological incubator. The bacterial colonies surrounding the plant parts were picked and streaked on fresh nutrient agar. (Kumar *et al*. 2015)

2.4. Extraction of antimicrobial compounds:

According to the modified methodology by Beiranvand *et al.,* 2017, the isolates were cultured in NB (Nutrient Broth) and incubated at 37°C for 14 to 16 days, after which the extraction of antimicrobial compounds was done. According to this method the cultured media was mixed with ethyl acetate in 1:1 ratio and stirred at intervals for 6 h. The organic supernatant was separated and centrifuged at 5,000 rpm for 10 min. The ethyl acetate layer was transferred into a clean flask, and dried at 50°C. The dry extract was dissolved in 2 ml of ethanol. To test antimicrobial susceptibility of the extracts, 25 µl of each extract in ethanol was used to add in the wells made in the agar. Bacterial isolates extracts obtained was tested by agar diffusion method against four foodborne pathogenic bacteria, *Salmonella typhi, Shigella spp., Enterococcus faecalis* and *E. coli.* The zone of inhibition (ZOI) was measured for each bacterial species separately (Beiranvand *et al.,* 2017).

2.5. Characterization of bacterial isolates

Endophytic bacterial isolates were characterized on the basis of colony morphology & biochemical characteristics. The morphological and biochemical characteristics of the isolates were examined according to the Bergey's manual of determinative Bacteriology (Kumar *et al*. 2015).

2.6. Phytochemical Analysis of the Bacterial Endophytes:

The Phytochemical analysis of the two bacterial isolates was done as per the following methodology in Table 1.

Phytochemical Test	Method	Characteristic Observation	Reference
Tannins	To 1 ml of extract, 2–3 drops of $FeCl_3$ were added.	Blackish-blue or blackish-green coloration	Harborne, 1998
Alkaloids	To 1 ml of extract, 2–3 drops of Dragendorff's reagent were added.	Turbidity or precipitation formation	Sofowora, 1993
Flavonoids	To 1 ml of extract, 2–3 drops of NaOH were added.	Yellow coloration	Harborne, 1998
Saponins	To 5 ml of extract, 2–3 drops of olive oil were added and shaken vigorously.	Stable froth or foam formation	Trease & Evans, 2002
Steroids	To 1 ml of extract, 1 ml $CHCl_3$ was added followed by 2–3 drops of conc. H_2SO_4.	Formation of reddish-brown ring	Sofowora, 1993

Table 1: Phytochemical analysis

3. RESULTS

The 16 bacterial endophytes isolated from the leaves, stem, bark and berries of *Coffea arabica* and *Coffea robusta* from Chikmagalur are as shown in Table 2. Two isolates CCRBI & CCRB2 from the barks of *Coffea robusta* were selected for further study and characterization based on their antimicrobial activity against the foodborne pathogens as detailed in Table 3. These isolates showed antimicrobial activity against *Shigella spp*., *S. typhi* & *E. coli* with large zones of inhibition compared to the other isolates. The morphological & biochemical characterization of CCRB1 & CCRB2 & the phytochemical analysis of the same are as shown in Table 4 & 5 respectively.

Sl. No.	Plant	Plant part	Organisms identified
1.	*C.arabica*	Leaves	CCAL1- Gram positive rods
2.	*C.arabica*	Roots	CCAR1- Gram positive rods
3.	*C. arabica*	Stem	CCASh1 – Gram positive rods

4.	*C. arabica*	Bark	CCAB1- Gram positive rods
5.	*C. robusta*	Leaves	CCRL1- Gram positive cocci CCRL2- Gram positive rods CCRL3- Gram positive rods
6.	*C. robusta*	Roots	CCRR1- Gram positive cocci CCRR2- Gram negative rods CCRR3- Gram positive rods
7.	*C. robusta*	Stem	CCRSh1- Gram positive rods CCRSh2- Gram positive rods
8.	*C. robusta*	Bark	CCRB1- Gram positive rods CCRB2- Gram positive cocci in clusters
9.	*C. robusta*	Berries	CCRSB1- Gram positive cocci CCRSB2- Gram positive rods

Table 2: Bacterial Endophytic isolates from *C. arabica* & *C. robusta* from Chikmagalur

CCAL: Chikmagalur *C.arabica* leaves, CCAR: Chikmagalur *C.arabica* root, CCASh: Chikmagalur *C.arabica* shoot, CCAB: Chikmagalur *C.arabica* bark, CCRL: Chikmagalur *C.robusta* Leaves, CCRR: Chikmagalur *C.robusta root*, CCRSh : Chikmagalur *C.robusta* shoot, CCRB: Chikmagalur *C.robusta* bark, CCRSB: Chikmagalur *C.robusta* seed/berry

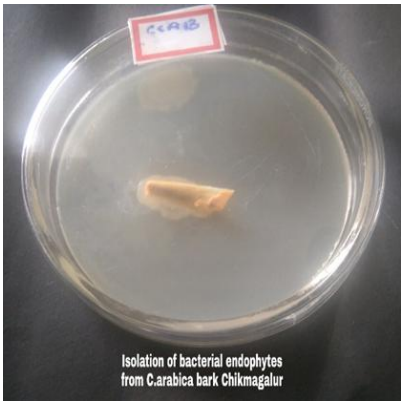


Figure 3: Bacterial Endophytic isolates from *C.robusta* & *C.arabica* from Chikmagalur

	Chikamaglur				
SL. NO.	**Bacterial Endophyte**	***Shigella dysenteriae***	***Salmonella. typhi***	***Enterococcus faecalis***	***E. coli***
1.	CCRL2	14.5 mm	-	-	-
2.	CCRL3	30.5 mm	-	-	-
3.	CCRSh1	20mm	25mm	-	20mm
4.	CCRSh2	25mm	-	-	18.5mm
5.	CCRB1	20.5mm	26.5mm	-	24mm
6.	CCRB2	20.5mm	21.5mm	-	28mm
7.	CCRR1	18mm	-	-	-
8.	CCRR2	23mm	16mm	13mm	-

Table 3: Actimicrobial activity of Bacterial endophytic isolates from *Coffea arabica* & *Coffea robusta* from Chikmagalur on Foodborne pathogens

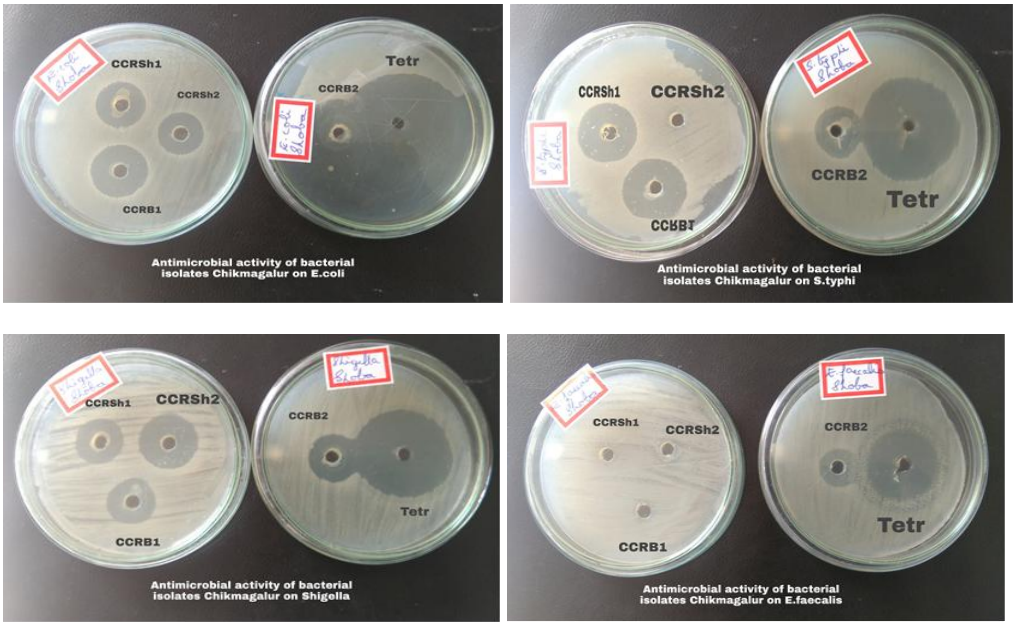


Figure 4: Antimicrobial activity of ethyl acetate extract of Endophytic bacterial isolates from Chikmagalur on Food borne pathogens:

Sl. No.	Bacterial Endophyte	Gram Character	Indole test	Methyl red test	VP test	Citrate utilization test	Catalase test	Oxidase test	TSIA test	Motility
1	CCRB1	Gram postive rod	-ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
2	CCRB2	Gram postive rod	-ve	+ ve	+ ve	+ ve	+ ve	- ve	+ ve	+ ve

Table 4: Morphological & Biochemical characterization of Bacterial Endophytic Isolates

Sl. No.	Bacterial Endophyte	Alkaloids	Tannins	Flavonoids	Saponins	Steroids	Reducing Sugar
1.	CCRB1	+ ve	- ve	+ ve	+ ve	- ve	- ve
2.	CCRB2	+ ve	- ve	+ ve	+ ve	- ve	+ ve

Table 5: Phytochemical analysis of Bacterial Endophytic isolates from Chikmagalur

4. DISCUSSION

In the present study, *Coffea arabica* and *Coffea robusta* were collected from plantations in Chikmagalur, the region historically recognized as the origin of coffee cultivation in India. A total of 16 bacterial endophytic isolates were obtained from different plant tissues of both coffee species. Interestingly, the number of isolates retrieved from *C. robusta* was higher compared to those from *C. arabica*, suggesting possible species-level differences in endophytic colonization.

The antimicrobial potential of all 16 isolates was evaluated against major foodborne pathogens, including *Escherichia coli*, *Enterococcus faecalis*, *Shigella* spp., and *Salmonella typhi*. Out of the total isolates, eight (50%) exhibited notable antimicrobial activity, highlighting the richness of coffee-associated endophytes as a reservoir of bioactive metabolites. Among these, two isolates designated as **CCRB1 and CCRB2**, both derived from the bark of *C. robusta*, demonstrated strong inhibitory activity against three of the four tested foodborne pathogens, with comparatively larger zones of inhibition. Due to their broad-spectrum activity, these two isolates were selected for detailed morphological, biochemical, and phytochemical characterization.

Phytochemical analysis revealed the presence of **alkaloids and saponins** in the crude extracts of CCRB1 and CCRB2. This observation is consistent with the reported phytochemical profile of coffee plants, which are rich in alkaloids

such as caffeine and trigonelline, along with saponins. Both alkaloids and saponins have been widely documented for their antibacterial properties, suggesting a possible correlation between host phytochemistry and its associated endophytic microbiota. The detection of these compounds in the endophyte extracts supports the hypothesis that microbial symbionts are capable of synthesizing bioactive metabolites similar to those of their host plants.
This investigation reinforced the potential of coffee-associated endophytes as promising candidates for antimicrobial drug discovery. Further purification and characterization of the crude extracts using chromatographic techniques, followed by structural elucidation, could provide valuable insights into novel antimicrobial compounds with pharmaceutical relevance.

4. STATEMENTS AND DECLARATIONS

AI Statement: The authors declare that they have used generative artificial intelligence, specifically ChatGPT and Copilot only for the creation of images.
Authorship Contribution: Dr. Manjunath: Conceptualization, funding; Ms. Florence Shoba: Carrying out the data collection, data curation, and writing the original manuscript and original draft.
Ethical Standards: All the ethical Research standards were followed while writing this conceptual paper.
Conflict of Interest: The authors state that they do not have any conflict of interest.
Informed Consent / Ethical Compliance: As this is a conceptual paper, there is need of any consent from anyone.
Human or animal involvement in the article: Nil
Data Availability: All data included in this research article is primary data of which references are provided.

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