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Biocontrol Activity of Yeasts against *Alternaria solani* and Plant Growth Promotion of Tomato Plants

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Yeasts have emerged as a single celled biocontrol agent which are applicable in controlling many disease causing pathogens in plants. These yeasts also have growth promoting substances that help in the growth and development of plants.
Materials and Methods: Yeast on tomato plants were isolated using standard plate count technique and observed under microscope for their cell shape and sizes. Biocontrol activity of yeast was evaluated by measuring diameter of zone of inhibition (mm) and estimating mycelial dry weights of pathogen. IAA production by yeasts was estimated *in vitro* and the plant growth and development was evaluated by measuring plant heights, number of leaves, number of days for flower initiation, shoot biomass, root mass, disease incidence and severity in pot culture experiments.
Results: Yeast isolate TPL-I isolated from tomato plants was most effective in inhibiting *Alternaria solani* with diameter of zone of inhibition of 9.00 mm. Reduced mycelial dry weight of pathogen *viz.,* 40.07 mg was exhibited by TPL-I under *in vitro* conditions. The maximum concentration of IAA was produced by TPL- I (0.11 µg/ml). The highest plant height, number of leaflets and number of days taken for flower initiation was recorded highest in T_4 (*Trichoderma harzianum*) followed by T_7 (Foliar spray of *Alternaria solani* + TPL- I + *Trichoderma harzianum*) at 30, 45 and 60 days after transplanting. Maximum dry weight of shoot (12.32 g/plant) and root (5.18 g/plant) was recorded in T_4 (Foliar spray of *Trichoderma harzianum*) whereas, minimum dry weight of shoot (8.26 g/plant)

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and root (2.80 g/plant) was recorded in T_2 (Foliar spray of *Alternaria solani*). Lowest disease incidence (33.33%) and disease severity (24.25%) was recorded in T_5 (Foliar spray of *Alternaria solani* + TPL- I).

Keywords: Yeasts; tomato; Alternaria solani; biocontrol activity; plant growth.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum*) belongs to the genus *Lycopersicon* under Solanaceae family. Tomato is an herbaceous plant growing to 1-3 m in height with weak woody stem. The flowers are yellow in colour and the fruits of cultivated varieties produce red fruits when ripe. According to Food and Agriculture Organization, the global production of tomato in 2020 was to the tune of 186.821 million metric tonnes on 5,051, 983 hectares land area. In India, according to National Horticulture Board tomato production was estimated to be 205.72 Lakh Tonnes in 2019-2020. Tomato is mainly grown as Rabi crop in the plains of India. Diseases usually occur, despite good management practices. The degree of occurrence is region bound and also largely depends on environmental conditions. Early blight of tomato, one of the dominant diseases in tomato caused by *Alternaria solani* fungi causes average yield loss of 35-78%. Symptoms of this disease include nectrotic spots in concentric rings with yellow chlorotic halo, which affect tomato plants by reducing its photosynthetic area [1].

Yeasts are group of fungi in which unicellular form are predominant. Yeasts are generally of three types namely Ascomycetous, Basidiomycetous and Deuteromycetous. Most of the yeasts are represented in Sub Division Ascomycotina and Basidiomycotina of the kingdom Mycota. Dimorphic yeasts are also present which become filamentous under certain environmental conditions [2].

Yeasts in general have varied distribution in the environment. They play important role in the dynamics of biological and chemical turnover in soil, plants, animals and water. They are generally present in the natural resources leaf surface, fruit juice, palm syrup, toddy, milk, soil, animal surfaces and in the intestinal tracts of warm-blooded animals, where they may live symbiotically or as parasites *etc* [3]. Yeasts are generally chemoorganotrophs as they use inorganic chemicals as sources of energy. They have various economic importance like in food fermentation, baking and biotechnological applications [4].

Few yeasts namely, *Candida famata, Pichia membranifaciens* and *Rhodotorula mucilaginosa* are known to have antagonistic activity. The antagonistic activity of yeasts are mainly based on properties such as competition for nutrients and space mycoparasitism, induction of host resistance, production of volatile substances (VOCs) and toxins [5]. The present investigation was aimed at obtaining effective strains of yeasts that have potential biocontrol activity against *Alternaria solani* which causes early blight of Tomato.

2. MATERIALS AND METHODS

2.1 Isolation of Yeasts

Yeasts were isolated from leaves, stems and fruits of tomato plants. The leaves were punched and leaf discs (30 leaf discs) were made. Small portions of stems and fruits were cut using sterile blades. All these samples of leaf discs, stem and fruit bits were suspended in 100 ml sterile distilled water blanks. After shaking for one hour, standard plate count technique was carried out for each samples separately [6]. Diluted samples of 0.1 ml were used for isolation of yeasts using Yeast Extract Malt Extract (YM) Agar Medium. The dilution 10^{-3} was used in the experiment. The plates were incubated at 28°C for 48 hours. The yeast cells was observed under microscope.

2.2 Biocontrol Activity of Yeast Isolates

2.2.1 Effect of different yeast isolates on growth of *Alternaria solani*

The effect of yeast isolates on the growth of *Alternaria solani* was evaluated. A suspension of *Alternaria solani* was mixed with molten (50°C) PDA medium contained in 500 ml flask so as to get a thick growth of fungi on the medium. The seeded medium was poured to petridishes and allowed to solidify. Sterilized filter paper discs (Whatmann No. 1) measuring of 7.0 mm diameter were impregnated with cultures of yeast

isolates was placed on the surface of seeded PDA contained in petriplates. The petriplates were incubated at 28°C for 7 days. The observations were recorded on the zone of inhibition produced around the filter paper disc by measuring the diameter of the inhibition zone (mm) [7].

2.2.2 Effect of yeast isolates on the mycelial dry weight of *Alternaria solani*

The nine yeast isolates were evaluated for their effect on mycelial dry weight of *Alternaria solani*. The 5 mm diameter of *Alternaria solani* maintained on the Potato Dextrose Agar medium (PDA) plates was transferred into 250-ml flasks containing 30 ml of the potato dextrose broth having yeast cultures and was kept on shaker at 180 rpm. After three days of incubation, the fungal mycelium was harvested by filtration through oven dried Whatman No. 42 filter paper. The mycelium was washed thoroughly with distilled water and then oven dried at 60°C for 3 days. The observations were recorded for the mycelial weights (mg) of *Alternaria solani* [8].

2.2.3 Evaluation of yeast isolates for Indole Acetic Acid (IAA) production

All the nine yeast isolates were evaluated for Indole acetic acid production (IAA) in *in vitro* conditions. Yeast cultures grown on YM agar plates (incubated at 25° C for 2 days) were used. Loop full of culture was inoculated to 5 ml of yeast extract peptone broth in test tubes, the cultured on the shaker at 30°C (150rpm) for 7 days. An aliquot of 1.5ml was centrifuged at 8000 rpm for 5 minutes. Supernatant was collected and one ml of Salkowski reagent (2 ml of 0.5M $FeCl_3$ in 49 ml water and 49 ml 70% perchloric acid) was added. After 30 minutes solution developed pink colour. The intensity of pink colour developed was read in spectrophotometer at 535 nm [9]. Standard curve was prepared with known concentrations of IAA. The quantity of IAA in the culture filtrate was determined using standard curve and expressed as µg/ ml of the medium.

2.2.4 Evaluation of selected yeast isolate for biocontrol of *Alternaria solani* and plant growth promotion of tomato in pot culture experiment

A pot culture experiment was carried out under greenhouse to study the effect of selected yeast isolates for biocontrol of *Alternaria solani* and plant growth promotion of tomato. Red loamy soil was collected and farmyard manure was added and mixed thoroughly and filled in plastic bags at the rate of 4kg per bag. The 28 days old tomato seedlings (Shivam tomato variety) were obtained from Hessaraghata, Bengaluru. The seedlings were transplanted on the same day. All the treatments were maintained in three replicates. Watering was done regularly.

The pathogen, *Alternaria solani* was grown on Potato Dextrose Broth (PDB) with constant shaking at 180rpm for 48 hours at room temperature and was given as foliar spray using a hand held spray bottle with 100 ml per plant at 30 Days After Transplanting (DAT). The yeast isolates and *Trichoderma harzianum* were also applied according to treatment to the plants. The cultures grown on PDB with constant shaking at 180rpm for 48 hours at room temperature was given as foliar spray using a hand held spray bottle at 30 Days After Transplanting (DAT). Observations on plant growth were recorded at 30 and 45 days after transplantation and at harvest. The plants were uprooted carefully and dipped in water for 30 minutes to loosen the soil. The plant height was measured from base of the plant to the tip of terminal leaf at 30, 45 and 60 days after transplantation. The numbers of leaflets on plants were counted on 30 DAT, 45 DAT and at harvest and were expressed as Number of leaflets per plant. The number of days taken to flowering from the date of sowing in each treatment was recorded [10].

Table 1. Treatment details

Treatments	Treatment details
T_1	Control (un-inoculated)
T_2	Foliar spray of *Alternaria solani* (pathogen)
T_3	Foliar spray of TPL- I (yeast isolate)
T_4	Foliar spray of *Trichoderma harzianum*
T_5	Foliar spray of *Alternaria solani* + TPL- I
T_6	Foliar spray of *Alternaria solani* + *Trichoderma harzianum*
T_7	Foliar spray of *Alternaria solani* + TPL- I + *Trichoderma harzianum*
T_8	Foliar spray of *Alternaria solani* + Mancozeb

2.2.5 Biomass of shoot and root

The biomass of plants were evaluated by recording the fresh and dry weight of the tomato plants in green house experiments.

2.2.6 Fresh weight of shoot and root (g)

The fresh weight of shoots and roots were recorded using electronic balance and mean was calculated to express the fresh weight of shoot and root in grams.

2.2.7 Dry weight of shoot and root (g)

The dry weight of shoots and roots were recorded after oven drying the sample at 60°C till it remained constant and mean was calculated to express the dry weight of shoot and root in grams [10].

2.2.8 Disease incidence of early blight of tomato (%)

The disease incidence (%) was assessed at 60 DAT on basis of symptoms occurred on the leaves which were black or brown lesions surrounded by a yellow halo during the growing season and were recorded by using the following formula:-

Disease incidence (%) = (Number of infected plants / Total number of plants assessed) x 100

2.2.9 Disease severity of early blight of tomato (%)

The disease severity was assessed on three leaves of randomly selected tomato plants at 60 DAT and the severity was scored using 0-9 points scale suggested by Mayee and Datar, [11].

3. RESULTS

The present work was carried out to isolate yeasts from leaves, stem and fruits of tomato plants. The yeasts were evaluated for their Indole acetic acid production and biocontrol activity against early blight causing fungi *Alternaria solani* under *in vitro* and in pot culture experiments.

3.1 Isolation of Yeasts

The yeast isolated from leaves, stem and fruits were observed under microscope and their cell shape and sizes were recorded (Table 3).

3.2 Effect of Different Yeast Isolates on Growth of *Alternaria solani*

Of the nine yeast isolates, TPL- I was found to be most effective in inhibiting the growth of the pathogen followed by TPS, RTPL, RTPF-I and RTPF- II producing maximum inhibition zone of 9.00mm, 1.00 mm, 1.00 mm 1.50 mm and 1.00 mm respectively. TPL- II, TPF, RTPS - I and RTPS- II were not effective in inhibiting the growth of the pathogen as no inhibitory zone was observed.

3.3 Effect of Yeast Isolates on the Mycelial Dry Weight of *Alternaria solani*

Among all the yeast isolates, highest reduction of mycelial dry weight (40.07 mg) of *Alternaria solani* was shown by yeast isolate TPL-I (Fig.1) followed by RTPF-I (101.17 mg) and RTPL (119.07 mg). RTPS-I was less effective in reducing the dry weight of *Alternaria solani* (211.10 mg).

Table 2. Level of resistance or susceptibity in tomato

Rating value	Nature of infection	Level of resistance or susceptibity
0	No symptom	Immune
1	Small circular, scattered, brown spots, covering 1 per cent or less of the leaf area	Highly Resistant
3	Spots enlarging, dark brown in colour covering 1 to 10 per cent of leaf area and infection on the lower most leaves of the plant	Resistant
5	Spots enlarging, dark brown in colour covering 11 to 25 per cent of leaf area and infection on the lower most leaves of the plant	Moderately Resistant
7	Spots dark brown in colour covering 26 to 50 per cent of leaf area and covering one third of the plant	Susceptible
9	Spots uniformly dark brown, coalescing, covering 50 per cent or more leaf area and severe infection on all leaves	Highly Susceptible

Table 3. Isolation of yeasts from leaves, stem and fruits of tomato plants

Yeast Isolates	Plant part	Colony character (on PDA medium)	Shape and size (µm)
TPL-I	Leaves	Pink, round	Oval=1-2 x1-3
TPL-II	Leaves	Dull white, round	Oval=1-3 x1-3
TPS	Stems	White, round	Oval=1-3 x1-2
TPF	Fruits	Dull white	Oval=1-4x1-4
RTPL	Leaves	Bright white	Oval=1-4x1-3
RTPS-II	Stems	Cream	Oval=1-3x1-4
RTPF-I	Fruits	White	Oval=1-3x1-2
RTPF-II	Fruits	Yellow	Oval=1-4x1-3
RTPS-I	Stems	White	Oval=1-3x1-2

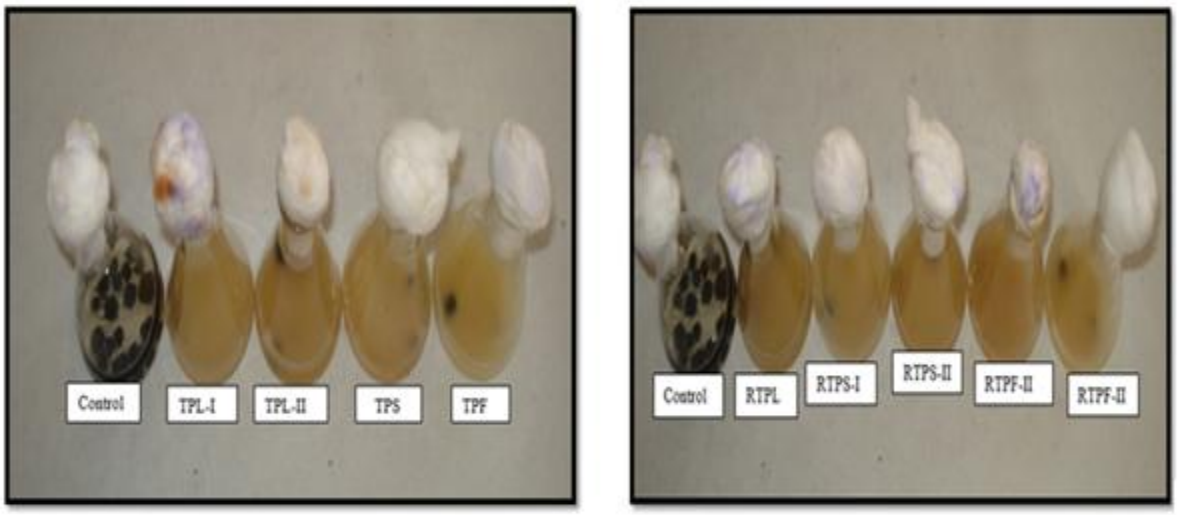

Fig. 1. Yeast isolates effect on the mycelial dry weight of *Alternaria solani*

3.4 Indole Acetic Acid (IAA) Production by Yeast Isolates

All the nine yeast isolates were screened for indole acetic acid production *in vitro*. Out of nine isolates, four yeast isolates produced relatively high concentrations of IAA (Table 4). Maximum IAA of 0.68µg/ml was produced by TPL- I yeast isolate and minimum IAA of 0.11µg/ml was produced by RTPF- I yeast isolate.

Table 4. Indole Acetic Acid (IAA) production by yeast isolates

Yeast isolates	IAA (µg/ml)
TPL-I	0.68
TPL-II	0.33
TPS	0.22
TPF	0.12
RTPL	0.17
RTPS-I	0.15
RTPS-II	0.13
RTPF-I	0.11
RTPF-II	0.21
Control	0.05
S.Em.±	0.01
C.D at 5%	0.02

3.5 Evaluation of Selected Yeast Isolate for Biocontrol of *Alternaria solani* and Plant Growth Promotion of Tomato in Pot Culture Experiments

Plant height was recorded at 30, 45 Days after transplanting (DAT) and at harvest for evaluation of the selected yeast isolate (TPL-I) for biocontrol of *Alternaria solani* and plant growth promotion in pot culture experiments.

The highest plant height was recorded in treatment T_4 (foliar spray of *Trichoderma harzianum*). After 30 and 45 DAT the plant height was 10.67 cm and 32.30 cm respectively. The lowest plant height of 10.03 cm and 28.90 cm was recorded in T_2 (pathogen sprayed) at 30 and 45 DAT respectively. At harvest, plant with foliar spray of *Trichoderma harzianum* in T_4 recorded highest plant height (53.87 cm) while the lowest plant height (44.77 cm) was recorded in T_2 (pathogen sprayed).

Number of leaflets at 30, 45 DAT and at harvest was recoded. At 30 and 45 DAT, more number of leaflets were recorded with T_3 (3.00 and 7.00 per plant). The minimum number of (2.67 and 4.33 per plant respectively) leaflets were found in T_2. At harvest, maximum number of leaflets were recorded in T_4 (13.67 per plant). However, minimum number of leaflets (9.67 per plant) was recorded in T_2.

The data on the number of days taken for flower initiation was also recorded. The minimum number of days (50.67 days) taken for flower initiation was in T_4 (foliar spray of *Trichoderma harzianum*), followed by in T_7 (foliar spray of Pathogen + TPL-I yeast isolate + *Trichoderma harzianum*) 52.00 days. The plants treated with pathogen (T_2) recorded maximum days for flower initiation (55.00 days).

3.6 Shoot Biomass (g/ plant)

At harvest fresh weight of shoot was maximum in T_4 with 25.20 g /plant. Dry weight of shoot was also maximum in T_4 (12.32 g / plant). The minimum fresh weight and dry weight of shoot (16.63 g and 8.26 g per plant respectively) was in T_2.

3.7 Root Biomass (g/ plant)

At harvest fresh weight of root was maximum in T_4 (8.53 g / plant. Dry weight of root was maximum in T_4 (5.18 g /plant). The minimum fresh weight and dry weight of root (5.17 g and 2.80 g /plant respectively) was in T_2.

3.8 Disease Incidence of Early Blight of Tomato (%)

The data regarding disease incidence (%) on tomato plants was recorded (Table 5). There was no disease incidence in the tomato plants of treatments T_1, T_3, T_4, T_6 and T_7.The lowest disease incidence (33.33%) was recorded in T_5 (foliar spray of pathogen + TPL-I) followed by in T_2 (pathogen) 66.66%.

3.9 Disease Severity of Early Blight of Tomato (%)

The disease severity (%) on tomato plants at 60 DAT was recorded (Table 6). There was no disease severity in the tomato plants of treatments T_1, T_3, T_4, T_6 and T_7. The lowest disease severity percent (24.25%) was observed in T_5 (foliar spray of pathogen + TPL-I) followed by in T_2 (pathogen sprayed) 47.67%.

Table 5. Disease incidence of early blight of tomato (%)

Treatments	Disease Incidence (%)
T_1- Control	0.00
T_2- Pathogen	66.66
T_3- Foliar spray of TPL- I	0.00
T_4- Foliar spray of *Trichoderma harzianum*	0.00
T_5- Foliar spray of pathogen + TPL- I	33.33
T_6- Foliar spray of pathogen + *Trichoderma harzianum*	0.00
T_7- Foliar spray of Pathogen + TPL- I + *Trichoderma harzianum*	0.00
T_8- Foliar spray of Pathogen + Mancozeb	0.00

Table 6. Disease severity of early blight of tomato (%)

Treatments	Disease severity (%)
T_1- Control	0.00
T_2- Pathogen	47.67
T_3- Foliar spray of TPL- I	0.00
T_4- Foliar spray of *Trichoderma harzianum*	0.00
T_5- Foliar spray of pathogen + TPL- I	24.25
T_6- Foliar spray of pathogen + *Trichoderma harzianum*	0.00
T_7- Foliar spray of Pathogen + TPL- I + *Trichoderma harzianum*	0.00
T_8- Foliar spray of Pathogen + Mancozeb	0.00
S.Em.±	0.52
C.D at 5%	1.55

4. DISCUSSION

In the present study, yeast isolate TPL-I isolated from tomato plants showed 9.00 mm diameter zone of inhibition and maximum reduction of mycelial dry weight of pathogen *Alternaria solani* (40.07 mg) and inhibition of conidial germination (80.50%) under *in vitro* conditions. Ciro et al*.* [12] reported the biocontrol activity of *Kluyveromyces* sp. $FP4_{13}$, isolated from samples of different frozen fruit pulps against the strains of *Penicillium expansum* and *Aspergillus ochraceus*. The yeast was most effective against the conidial germination of the pathogens, showing inhibition rates of 93.33 and 86.44% for *P. expansum* and *A. ochraceus*, respectively. And the mycelial growth inhibition was 28.45 and 21.0%, respectively. Four yeast isolates produced relatively high concentration if IAA in the range of 0.21 - 0.68 µg/ml. The maximum concentration of IAA was produced by TPL- I (0.68 µg per ml). Deng et al*.* [13] reported on yeast *Cryptococcus* sp. CBSB78 which was isolated from rape *(Brassica chinensis*), although producing IAA concentrations of 11.7 µg per ml and promoted the growth of *Brassica* sp. in metal contaminated soils.

Maximum plant height was seen with the foliar spray of *T. harzianum* (53.87 cm) which is true in accordance with the findings of Nzanza et al*.* [14] who observed such increased plant height in tomato crop with *Trichoderma harzianum* as a bioagent. And the results of increased plant height by yeast isolate may be attributed due to the cell contents having different nutrients, higher percentage of proteins, higher values of vitamins, especially Vitamin B which may play an important role in improving growth and controlling the incidence of fungi diseases. Maximum leaflets were recorded with foliar spray of *T. harzianum* as reported by Ravindra et al*.* [15] who observed the increase of total number of leaves in tomato when the crop is amended with *T. harzianum* + soil treatment with neem cake powder + foliar spray with carbendazim. Significantly less number of days was taken for tomato flower initiation in T_4 (Foliar spray of *Trichoderma harzianum*) 50.67 days, followed by in T_7 (foliar spray of Pathogen + TPL-I + *Trichoderma harzianum*) 52.00 days and T_3 (selected yeast isolate TPL- I) 52.33 days. Uddin et al*.* [16] reported *Trichoderma* produces auxins, more chlorophyll content and carotenoids in tomato that are able to stimulate flowering and plant growth and development. Foliar application of tomato plants with yeast isolate had significant differences in shoot and root biomass of tomato. These results are in agreement with the findings of Roshan et al*.* [17] who found that the initial lesions on tomato leaves expand and new lesions develop causing the entire leaves to turn chlorotic leading to significant defoliation and thereby reduce the overall plant and development.

The disease incidence and disease severity on tomato plants was less in T_5 (foliar spray of pathogen and TPL- I) 33.33% and 24.25% respectively. Whereas, the tomato plants sprayed with *Alternaria solani* in T_2 recorded highest disease incidence of 66.66% and disease severity of 47.67%. The results are in agreement with Sathiyabama, et al*.* [18] who observed low level of disease severity caused by *A. solani* with the foliar sprays of yeast extract glucan which induce higher chitinase activity that plays a major role in the restriction of the pathogen (*A. solani*) in tomato plants.

5. CONCLUSION

The yeast isolate TPL-I isolated from tomato plants was effective in its biocontrol activity against *Alternaria solani*. It formed maximum zone of inhibition (9mm) and produced minimum dry mycelial weight (40.07 mg) of pathogen. Among the other yeast isolates, four yeast isolates produced relatively higher concentrations of IAA ranging from 0.21 - 0.68 µg/ml. The maximum concentration of IAA was produced by TPL- I (0.68 µg per ml). Foliar application of the best yeast isolate (TPL- I) and *Trichoderma harzianum* significantly increased plant growth of tomato and decreased the disease incidence and disease severity of *Alternaria solani*. Thereby, these treatments could be effectively used in the biological control of *A. solani* and also plant growth promotion of tomato.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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