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A consortium of thermophilic microorganisms for aerobic composting

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Abstract: *Accumulation of waste materials is a major crisis in the present world causing increased environmental threat. Aerobic composting is the process of conversion of solid waste materials into stable compost by the activity of aerobic microorganisms. Thermophilic microorganisms involved in aerobic composting, especially which encompass elevated ability for the production of waste degrading enzymes play a superior role in escalating the degradation rate. A consortium of 14 different thermophilic microorganisms which is having the maximum enzyme production was selected for composting weeds, herbal medicinal waste materials, coir pith and sawdust. The consortium could increase the rate of degradation by 30 to 40 % for weed waste, herbal medicinal waste and coirpith. In the case of sawdust also the selected consortium could reduce the C/N ratio, but could not make final quality compost.*

Keywords**:** *composting, thermophilic microorganisms, consortium, enzymes, C/N ratio*

I. Introduction

Aerobic composting is a thermogenic solid state fermentation process, where the initial stage of composting is characterized by the activity and growth of mesophilic microbes. The microbial activity leads to rapid increase in temperature inside the waste material and in the next stage thermophilic microbes become responsible for the degradation process [1]. Analysis of microflora and its transition during the course of composting processes will provide important clues that help to overcome the problems encountered during microbial methods of recycling garbage [2]. The study of different physical, chemical and biological parameters including enzymatic activities associated with composting will give a better insight into the biological process involved and the quality of finished compost [3]. The objective of the present study is to provide information regarding the characterization of the enzymes produced by the thermophilic microorganisms and suggest on how they could be used for improving composting process.

II. Materials and Methods

2.1. Collection of samples

Compost samples were collected from five different organic materials which were either undergoing aerobic composting or biodegradation. Samples were drawn (1) from partially composted mixed weed with *Eupatorium odoratum* as the major plant species (2) herbal waste from medicine manufacturing factory, Oushadhi located at Thrissur, (3) tea waste compost originated from tea leaf waste produced after extraction of instant tea factory located at Munnar in Idukki district, (4) coir pith sample comprised of incompletely decomposed coconut husk from which fibers were removed and (5) saw dust comprised of partially decomposed saw dust which accumulated in saw mills for several months. The temperatures inside the compost heap of the first three samples were confirmed as 55-70^{0}C at the time of collecting the samples. Samples were collected from different points inside each heap of the above five sources. The moisture content of the samples was estimated**.**

2.2. Isolation of thermophilic microorganisms

Serial dilution plate technique was adopted for the enumeration and isolation of bacteria, actinomycetes and fungi using spread plate method. Nutrient agar was used for the enumeration of bacteria, starch casein agar for actinomycetes and Rose Bengal agar for fungi. 0.1 ml of 10^{-4} dilution was used for the isolation of fungi and actinomycetes and 10^{-5} dilution for bacteria. The agar plates were incubated in an inverted position at 50^{0}C in an incubator. Two days incubation was given for bacterial isolates, one-week incubation for fungal isolates and ten days incubation for actinomycetes. Five replica plates were inoculated for the isolation of the organisms.

2.3. Screening of microorganisms for further studies

The microbial colonies from the plates were purified by sub culturing them to new plates containing the respective media for bacteria, actinomycetes and fungi. The colonies were screened for the colony morphology

and microscopic analysis. All the isolates were also characterized by evaluating catalase, amylase, cellulose, xylananse and phenol oxidase production ability of the isolates.

2.4. Screening the isolates for enzyme production

For testing catalase production ability, the isolates were analyzed for the production of effervescence on 3 per cent hydrogen peroxide. Based on the quantum of the bubbles, the efficiency of the cultures was graded. The appearance of clear zone around the microbial growth when flooded with Lugol's Iodine was taken as the hydrolysis of starch done by the amylase enzyme. The appearance of clear zone around the colony showed the production of cellulase enzyme when colonies grown on cellulose agar medium were stained with 0.1 per cent congo red solution for 10min and then de-stained with 1M NaCl solution for half an hour. Xylanase enzyme production was analyzed on xylan agar medium by staining with 1 per cent congo red solution for 10 minutes and then de-staining using 1N NaOH solution. The method of Giltrap, (1982) was used to determine the ability to degrade soluble phenolics by phenoloxidase enzyme. Microorganisms capable of degrading tannic acid turn the color of the MMN agar from pinky grey to brown through 'Bavendamm reaction'.

2.5. Enzyme quantification

All the isolates which have been isolated in pure culture were tested for the enzyme production in plate assay. Three isolates, viz: *Bacillus subtilis* (Wb2), *Streptomyces albicans* (Ca2) and *Streptoverticillium reticulum* (Fa6) having the highest enzyme activity observed in plate assay were selected for quantitative estimation of amylase, cellulase and xylanase respectively in fermenter (Lark Innovative, Coimbatore, Tamil Nadu) after precipitation and purification.

Bacillus subtilis was inoculated into the production medium i.e Czapek Dox broth with 3 per cent starch for the production of amylase. For cellulase, *Streptomyces albicans* was inoculated to Bergs broth with 3 per cent Carboxy methyl cellulose and *Streptoverticillium reticulum* was also inoculated into Bergs broth with 3 per cent xylan for xylanase production. The pH of the medium was maintained at 7, temperature 50^0C for 72 hours and stirring at 120rpm. After incubation period, the saturated production media were spun in a centrifuge at 8000rpm at 4^0C for 15min. The supernatant of each broth was precipitated using ammonium sulphate.

The precipitated samples of amylase, cellulase and xylanase were loaded into separate dialysis bags (HIMEDIA, Bombay) and the contents were dialyzed with 1mM Tris HCl buffer (pH 8) by frequently changing the buffer every 5 to 6 hours at 4^0C. The total protein and enzyme activity of the three samples were measured by the procedure of Lowry et al., (1951) and dinitro salicylic acid method respectively.

2.6. Selection of microorganisms for consortium

Fourteen isolates which are non-antagonistic [6] against each other were selected for testing their suitability for preparing the consortium of microorganisms to be used for enhancing the speed of composting. The selection was based on growth rate and the efficiency of the isolate to produce specific enzymes which are having definite role in biodegradation. The microorganisms showing maximum enzyme activities and growth rate were selected as the suitable isolates for increasing the composting rate of substrate. These isolates were mass cultured in suitable culture broth and incubated in an incubator shaker for 5 days at 50^0C. The turbid broth cultures were analyzed for the microbial count by dilution plate technique before testing the efficiency.

2.7. Testing the isolates for composting efficiency

Four waste raw materials were selected for testing the efficiency of the isolates in enhancing speed of composting and compost quality. These were weed materials, Ayurvedic herbal waste materials, coir pith and saw dust. Weed waste were collected from Kerala Forest Research Institute campus, chopped to 1-2.5 cm size and inoculated with a mixture of equal quantity (cfu) of fourteen selected isolates at the time of initial stacking. In the case of herbal waste materials, the waste materials were collected from Ayurvedic (Oushadhi) medicine factory at Kuttanellur, Thrissur. Coir pith was collected from co-operative coir factory, Alpara, Thrissur and saw dust from saw mills at Paravattani, Thrissur.

Three replicates were kept for testing the activity of the consortium in each of the substrate. The aerobic composting was carried out in 35L capacity plastic buckets provided with sufficient numbers of holes for proper aeration; $3/4^{th}$ of the buckets were filled with the waste materials. Water was sprinkled to the waste materials so as to maintain 50-55 per cent moisture content. The consortium of the 14 cultures which contained a microbial count of $5x10^4$ to $5x10^5$ cells per ml for each culture was added to the waste materials as inoculum. Every day, the temperature inside the composting material was measured and when the temperature was above 45^0C the compost was aerated. Maintenance of moisture content, measurement of temperature and aerating the samples were continued for three to four weeks. The finished composts were used for further analysis.

2.8. Chemical analysis of the compost

The sample was analyzed for the mineral components, the moisture content and the type of the microorganisms active in each of the compost samples. The total carbon, hydrogen and nitrogen components of the compost samples were estimated using CHNSO elemental analyzer (Elementar Vario ELIII). The moisture content of the sample was measured in the initial phase of composting and also in the final matured stage. The population count of the microorganisms in the materials undergoing composting was tested using dilution plate technique on 10^{th} and 20^{th} day of composting.

III. Results

3.1. Moisture content of the samples

The moisture content of the five samples from which consortium of microorganisms were isolated showed variation. Partially degraded saw mill waste showed maximum moisture content (60%); the weed (57%) and Oushadhi waste (52%) also had high moisture content. Coir pith (42%) and Tea waste (46%) had lower moisture content compared to the other three composting materials.

3.2. Microbial population

The colony counts obtained in the serial dilution method are provided in Table 1. The mean number of colony forming units (cfu) of bacterial, actinomycete and fungal colonies were significantly different among the five samples.

Table 1: Average microbial population (cfu) per gram dry weight of the sample

Sl. No.	Samples	Bacteria (cfu/gm dry weight)	Actinomycetes (cfu/gm dry weight)	Fungi (cfu/gm dry weight)	Total (cfu/gm dry weight)
1	Weed compost	99.2×10^5	58.6×10^4	65.2×10^3	105.7×10^5
2	Ayurvedic herbal waste compost	20.2×10^6	96.6×10^4	91.7×10^3	212.6×10^5
3	Tea waste compost	34.6×10^4	12.8×10^5	43.6×10^3	16.7×10^5
4	Coir pith	45.6×10^3	12.2×10^3	24.4×10^2	82.2×10^3
5	Saw dust	12.6×10^3	10.6×10^3	48.5×10^2	28×10^3
6	ANOVA computed F value	794.53*	824.45*	467.41*	
7	Tabular $F_{(4,20)}$ at 5% level of significance	2.87	2.87	2.87	

*Significant at 5% level

3.3. Bacterial, actinomycete and fungal isolates selected for detailed study

The identical cultures from the same source or from different sources were eliminated by selecting only one representative isolate from identical colonies, having the same morphological characters and biochemical characters for detailed characterizaion. Thus, 128 isolates (39 isolates from weed waste, 22 from factory waste, 13 from tea waste, 33 from coir pith waste and 21 isolates from saw dust waste)were screened finally for detailed characterization.

3.4. Plate assay for enzyme production

Majority of the isolated organisms showed high level of enzyme production (Table 3).

Table 2: Percentage (%) of enzyme producing microorganisms from the five sources

Microorganisms	Amylase	Cellulase	Catalase	Phenol oxidase	Xylanase
Bacteria	61.2	69.4	95.9	63.3	38.8
Actinomycetes	95.2	96.8	100	67.7	93.5
Fungi	98.2	94.1	100	70.6	88.2

3.5. Enzyme activity estimation

The protein content, enzyme activity and specific activity of each of the enzyme were calculated and presented in Table 4. *Bacillus subtilis* (Wb2) showed the maximum specific activity.

Table 3: Protein content, enzyme activity and specific activity of amylase, cellulase and xylanase

Species tested	Enzyme	Protein content	Enzyme activity	Specific activity
Bacillus subtilis (Wb2)	Amylase	2.1mg/ml	600U/ml	285.7U/mg of protein
Streptomyces albicans (Ca2)	Cellulase	1.98mg/ml	500U/ml	252.5U/mg of protein
Streptoverticillium reticulum (Fa6)	Xylanase	1.92mg/ml	200U/ml	104.2U/mg of protein

3.6. Selection of isolates

From the 128 microbial cultures, 14 cultures were selected to form the consortium based on their ability for the production of enzymes (Table 5). From the enzyme analysis, it was concluded that actinomycetes were more efficient in enzyme production than fungi and bacterial isolates. Hence, for the final selection of isolates to form suitable consortium, more numbers of actinomycetes were included in the test.

Table 4: The list of species selected for testing their suitability to develop consortium

S. No	Isolate	Isolate No.	Species
1	Bacteria	Wb2	*Bacillus subtilis*
2		Cb6	*Bacillus stearothermophilus*
3	Fungus	Cf3	*Humicola sp*
4		Sf1	*Aspergillus sp.*
5		Wf1	*Aspergillus fumigatus*
6	Actinomycetes	Wa9	*Streptomyces celluloflavus*
7		Ca2	*Streptomyces albicans*
8		Wa6	*Streptomyces purpureus*
9		Ca6	*Streptomyces sulfonofaciens*
10		Sa7	*Streptoverticillium viridoflavum*
11		Fa6	*Streptoverticillium reticulum*
12		Wa8	*Streptoverticillium cinnamoneum*
13		Ca3	*Thermomonospora alba*
14		Wa3	*Thermomonospora curvata*

3.7. Testing the consortium

A period of three weeks of composting resulted in good compost in the case of weed and herbal waste (Table 6). However, coir pith compost took 26 days to get satisfactory compost. In the case of saw dust, composting did not progress after two months incubation.

The temperature of the materials undergoing composting was recorded daily. In the case of weed and herbal waste, the temperature inside the compost heap increased beyond 50^0C while in the control buckets and in the buckets filled with coir pith and saw dust the temperature was below 40^0C. The temperature inside weed compost stabilized at 30^0C on 19^{th} day. All the usual activities such as turning for aeration and maintaining sufficient moisture content (55%) were done once in 3 days. The turning was stopped when the temperature inside the compost was close to ambient temperature (Table 6).

Table 5: Effect of inoculation of consortium of thermophilic microorganisms on composting speed

Compost sample	Max temperature on 4^{th} day (^{0}C)	No of days taken to complete composting	No of turnings carried out
Weed compost	50	19	9
Ayurvedic factory herbal waste compost	51	22	9
Coir pith	40	26*	9
Saw dust	40	50*	9

*Temperature inside the compost heap fell down below 40^0C after 22 days

3.8. Mineral components

Table 7 shows the total per cent of C, N and S content and C/N ratio of the final compost. The per cent of total nitrogen, carbon and sulphur components varied in all the four samples. The highest percentage of total nitrogen was shown by weed compost (3.77%) closely followed by Oushadhi herbal waste compost (3.57%); the saw dust showed the lowest percentage of N (0.14%). The C/N ratio was lowest for weed compost (9.78) while the ratio for herbal waste compost was 10.31. Coir pith had a C/N ratio of 18 which is acceptable for coir pith compost. But in the case of saw dust the C/N ratio was 275. The consortium was found unsuitable for sawdust (Table 7).

Table 6: The mineral components of the final compost

S. No	Sample Name	C%	S%	H%	N%	C/N	Initial C/N ratio of waste materials
1	Weed compost	36.87	0.47	5.55	3.77	9.78	20-45
2	Ayurvedic herbal compost	36.81	0.30	5.78	3.57	10.31	35-245
3	Coirpith compost	11.92	0.14	9.34	0.64	18.63	100-150
4	Sawdust compost	38.57	0.14	6.85	0.14	275.5	400

3.9. Microbial population count

The microbial population counts per gram of the four samples drawn on 10^{th} day of composting were estimated. Table 8 gives the number of bacterial, actinomycetes and fungal colonies surviving in the compost samples. The total number of actinomycetes was found to be higher compared to the bacterial and fungal isolates. Identity of individual colonies of bacteria, actinomycetes and fungi was determined based on the morphological features. It was found that *Streptomyces celluloflavus, Streptomyces albicans, Streptoverticillium viridoflavum, Streptoverticillium reticulum, Bacillus subtilis and Humicola sp.* were found in higher numbers than the other species in the consortium. Table 9 gives the list of species which was finally concluded to be best as consortium selected based on the competitiveness of the isolates during composting process and their biochemical characteristics.

Table 7: Colony count of the consortium of microorganisms present in the materials undergoing composting on 10^{th} day

S. No	Samples	Actinomycetes (cfu/gm)	Bacteria (cfu/gm)	Fungi (cfu/gm)
1	Weed compost	53×10^6	97.2×10^4	3.2×10^2
2	Ayurvedic herbal waste compost	47.2×10^6	93.6×10^4	2.2×10^2
3	Coir pith compost	56.2×10^6	23.2×10^4	2.8×10^2
4	Saw dust compost	44×10^6	87.2×10^4	5.4×10^2

Table 8: List of species selected as best for consortium

S. No.	Isolate No.	Species
1	Sa7	*Streptoverticillium viridoflavum*
2	Fa6	*Streptoverticillium reticulum*
3	Wa9	*Streptomyces celluloflavus*
4	Ca2	*Streptomyces albicans*
5	Wb2	*Bacillus subtilis*
6	Cf3	*Humicola sp.*

IV. Discussion

In the present study, five different compost sources undergoing composting/biodegradation process were compared in respect of thermophilic microbial populations per gram dry weight of the sample. The comparison of thermophilic microbial population showed significant difference between the microbial populations existing in the five composting samples. There are several parameters which affect the microbial biomass during composting process. The moisture content, pH, and C/N ratio of the materials influence the microbial properties in a compost sample [7, 8]. A C/N ratio of 25-35 and moisture content between 50-55 per cent are optimum for good proliferation of microorganisms in compost [9, 10]. The mixed ayurvedic herbal factory waste had a C/N ratio of 35.3 and moisture content of 52 per cent, which supported largest microbial population. Even though, the C/N ratio was optimum in weed waste (27), the reason for the higher microbial population in herbal waste could be the residues of other nutrients such as jaggery, grains, etc. which are usual components of herbal medicine. The microbial count in weed waste was found to be moderately higher than the microbial count in tea waste, coirpith and saw dust samples. Weeds are good raw material for aerobic composting and the suitable C/N ratio (27) also supports the successive growth of microorganisms [9, 11].

Tea waste also has a C/N ratio of 27 and 42 per cent moisture. The lower moisture content and the presence of higher quantity of phenolics in tea leaves could be the other reason for the reduced multiplication of microbes in tea waste. Oxidized polyphenols have inhibitory activity against bacterial growth [12].

In the case of coir pith, the C/N ratio is 100 and moisture content 46 per cent, both of which cannot support the growth of microorganisms efficiently and hence the microbial count was comparatively less than that of the microbes in weed waste and ayurvedic herbal waste. Sasirekha and Rajarathnam, (2007) also reported that due to the high lignin content (~48%) and amorphous powdery nature, coir pith supported poor microbial growth.

In the case of saw dust, the C/N ratio is found to be 400 and the moisture content is 60 per cent. Sawdust showed the lowest microbial count i.e. 28×10^3 cfu per gram. Even though the moisture content is

conducive for the proliferation of microorganisms, the high C/N ratio (>400) could be the main reason for the reduction in the microbial biomass. Chung Tang et al., (2003) have reported that the wood compost had less microbial biomass which is due to the presence of resistant substrates like lignin. Chandra, (1999) observed that for the average C/N ratio in the range 25-30, the moisture content should be maintained at 50 to 60 per cent for the microorganisms to grow efficiently.

Even though there are many studies which support that C/N ratio is the major factor affecting the multiplication of microorganisms in compost, from the present study it can be concluded that the C/N ratio is not the only factor which is scheming the thermophilic microbes in compost. According to Ishii and Taaki, (2003), the analysis of microbial community in different compost products namely dehydrated sewage sludge mixed with sawdust, dehydrated sewage sludge mixed with woodchips, food waste mixed with sawdust, and food waste alone showed variation despite the similar C/N ratios. It has been concluded that despite the similar C/N ratio, the microbial proliferation is influenced by the qualities of the composting material.

Actinomycetes are commonly found during the thermophilic stage of composting [16]. Actinomycetes are an important part of the composting microbial community because they play a larger role in converting hemicellulose and cellulose to more readily degradable substrates such as starch and sugar [17]. Hence, it is essential to promote actinomycete colonization and activity for fast and efficient composting.

The fungal population in the current study was found to give the lowest plate count when compared with the bacterial and actinomycete populations. Mixing the compost materials, which result in disrupting hyphae or inhibiting the sporulation of members of the fungal community during composting [18] would have restricted the fungal growth to the maximum. Hoitink and Boehm, (1999) pointed out that fungal growth is favorable after the thermophilic phase in a composting process. In the present study, the original sample used for isolation was collected from compost heap during thermophilic condition to isolate all the organisms. Hence the reduction in fungal growth is justified.

In aerobic composting process, the microorganisms should be able to produce specific extra cellular enzymes and be able to grow at high temperatures [20]. In the present study, the microorganisms isolated from partially composted lignocellulose materials were screened for the production of five important enzymes, namely: amylase, cellulase, catalase, phenol oxidase and xylanase. The results of screening for the production of enzymes showed that the actinomycetes possessed the highest ability to produce all the five enzymes compared to that of the fungal and other bacterial isolates. It is reported that when the temperature rises above 30°C, actinomycetes, in particular S*treptomyces*, thrive and play an important role in composting by degrading natural polymers and colonize organic materials after bacteria and fungi consume easily degradable fractions [21]. Cellulose and hemicellulose originating from plant material, and lignin and humus are the chief carbon and nitrogen sources for actinomycetes [22]. There is also increasing evidence of the ability of actinomycetes to degrade xenobiotic compounds [23].

Fungal isolates had a higher ability to produce phenol oxidase (70.6%) compared to bacterial (63.3%) and actinomycete (67.7%) isolates. Cooney and Emerson, (1964) emphasized on the fact that the fungi are particularly well equipped with the enzymes necessary for decomposing complex plant materials, and are the conspicuous component of microflora of most self heating, composting plant material.

In the present study, *Bacillus* species showed higher ability of production of majority of the specific enzymes than other species. Lonsane and Ramesh, (1990) observed that cultures producing thermo-stable alpha amylase was limited to the genus *Bacillus*. Organisms capable of producing higher quantity of enzymes can be utilized for the development of a suitable consortium for the aerobic composting of organic waste materials. Bacteria are responsible for most of the initial decomposition and heat generation in compost, provided that the major growth requirements are met [26].

A consortium of microorganisms has been developed by testing the 14 isolates in the four raw materials, in such a way that the same can be used for composting a variety of raw materials. So, the most suitable isolates will be those isolates which are having the ability to decompose a variety of substrates. In the present study, the maximum enzyme producing isolates were found to be actinomycetes. The test consortium of microorganisms has 9 actinomycetes, 3 fungi and 2 bacterial isolates. But only six isolates comprising two *Streptomyces* sp, two *Streptoverticillium* sp; one *Bacillus* sp. and one *Humicola* sp. were finally recommended for inclusion in the consortium. For bringing down the number of organisms to six in the final consortium, the practical problem of using 14 organisms and the higher competitiveness of the selected six organisms were considered. *Humicola* sp. was included in the final consortium because of its high performance in biochemical characterization. Fungal consortium of *Aspergillus* and *Humicola sp.,* and actinomycetes, especially *Streptomyces* had been earlier used for the conversion of nutrient rich compost [11, 27].

The carbon, nitrogen and sulphur analysis of the compost samples obtained using the consortium gave an idea about the quality of the compost samples produced. The C/N ratio of the compost which gives a major insight into the quality of the compost was also calculated for all the four compost samples obtained after composting test. The C/N ratio of weed compost was found to be 9.78 and that of herbal waste compost was

10.31, indicating the high quality of composts. For coir pith, the selected consortium was found suitable to reduce the C/N ratio from 100-200 to 18.6. The coir pith compost had a slightly lower quality to that of the weed and herbal factory waste compost. But the final product had a dark color and earthy odor typical of good quality compost. The C/N ratio of weed compost, ayurvedic herbal waste compost and coir pith compost showed that these three composts produced with the help of the consortium had a C/N ratio indicative of good quality compost. But in the case of saw dust the C/N ratio was too high (275) and hence it was concluded that the consortium developed would not be suitable for the development of saw dust compost.

The ideal C/N ratio of the raw materials for composting is generally considered to be around 30:1 [28]. But the C/N ratio of saw dust is 100-500:1. The higher C/N ratio indicated that sufficient quantity of nitrogen for optimal growth of the microbial populations is unavailable in saw dust; so the compost remained relatively unaffected and the degradation took place in a very slower rate [29]. For quicker degradation of saw dust sufficient quantity of nitrogen has to be added so as to drastically decrease the C/N ratio.

V. Conclusion

Conversion of solid waste materials to useful products remains as a challenge even today. Composting is accepted to be one of the methods for converting waste materials to eco-friendly products. However, there is scope for further improving the technology. The present study reports development of a consortium of microorganisms comprising bacteria, fungi and actinomycetes for quick aerobic composting. Since the selection of the microorganisms is based on production of five important enzymes capable of degrading major components of organic waste materials, the consortium can be applied to wide range of organic waste materials. The application of the consortium is expected to bring out higher quality compost within a shorter period of time from organic waste materials with a C/N ratio less than 100. Even though, the lower efficiency of the consortium on substrates with C/N ratio higher than 100 is a limitation, the substantial reduction of C/N ratio found in the final product is a positive indication. Application of the consortium helps not only to reduce time of composting, cost of production and chances of pathogenic contamination, but also to quickly develop quality compost.

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