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Isolation, Morphological and Biochemical Characterization of Potassium Solubilizing Bacteria (KSB) Isolated from Northern Part of Karnataka

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The systematic survey was made to isolate efficient strains of potassium solubilizing bacteria from different crop plant rhizosphere such as Sorghum, Maize, Bajra, Cotton, Sunflower and Groundnut. A total of 75 isolates were isolated, based on the results obtained from *in vitro* studies 28 best isolates were selected. The diameter of zone of solubilisation formed by isolates ranged from 0.30 cm to 1.40 cm at 72 hours after incubation. The isolate, KSBD-58 showed maximum solubilisation zone of 1.40 cm, which was followed by KSBR- 41 (1.30 cm). Based on the morphological characterization it was revealed that out of 28 isolates 25 were *Bacillus* and five were *Pseudomonas.* With respect to biochemical characterisation all isolates tested positive for catalase, urease and acid production and negative for voger proskauer, denitrification and H_2S production test.

Keywords: KSB, *Bacillus, Pseudomonas, Fratueuria aurantia.*

Potassium is one of the essential macronutrient and the most abundantly absorbed cation in higher plants. It plays an important role in the growth and development of plants. In addition to plant metabolism, potassium improves crop quality because it extends the grain filling period, helps with grain filling and kernel weight, strengthens straw, increases disease resistance, and also helps the plant to withstand stress. Indian soils have sufficient quantity of potassium to support crops raised in it, but ironically, this potassium content in soil is in unavailable form to plants. Identifying a suitable solution to convert this unavailable form of potassium (which accounts for 90 to 98 % of total potassium in all soils) in to available form can resolve this problem and will promote balanced nutrition and sustainable soil health. Potassium Solublizing Bacteria (KSB) would be a novel solution to convert insoluble form of soil potassium into soluble (available to plant) form. Potassium solubilizing bacteria such as *Frateuria aurantia, Bacillus mucilagenosus* and *Bacillus edaphicus* are example of microorganisms that are used as potassium biofertilizer. Potassium solubilizing bacteria are able to solubilize potassium rock through production and secretion of organic acids (Han and Lee, 2005). They can enhance mineral dissolution rate by producing and excreting metabolic by-products that interact with the mineral surface. Mineral potassium solubilization by microbes which enhances crop growth and yield when applied with a cheaper source of rock potassium may be agronomically more useful and

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environmentally more feasible than soluble K (Rajan *et al.*, 1996).

Application of chemical fertilizer have side effects, such as leaching out, polluting water basins, destroying microorganisms and friendly insects, and making the crop more susceptible to the attack of diseases. Thus, biofertilizer is more favourable and encouraging to be used for its eco-friendly nature. Therefore, to increase the quality of biofertilizer at least as good as chemical fertilizer, inoculation of efficient KSB is necessary to increase potassium uptake on plants.

Considering the above, an attempt was made to isolate the efficient KSB isolates from different regions of North Karnataka.

MATERIALS AND METHODS

The present investigation was carried out at the Department of Agricultural Microbiology, College of Agriculture, Raichur for isolation, characterization and screening of potassium solubilizing bacteria isolated from rhizosphere of different crops grown in Lingasugur, Raichur and Deodurga taluk.

Isolation of Potassium Solubilizing Bacteria (KSB)

A total of 75 rhizosphere soil samples of different crops were collected from Lingasugur, Raichur and Deodurga taluk for isolation of KSB strains. Potassium solubilizing bacteria were isolated from rhizosphere soil samples by serial dilution plate count method using Aleksandrov medium (Hu *et al.*, 2006) which is a selective medium for isolation of potassium solubilizers supplemented with mica. The inoculated plates were incubated at room temperature of 30±1 °C for 3 days, the colonies exhibiting clear zones were selected and diameter of the zone was expressed in cm, and the colonies were purified by four way streak plate method and preserved on agar slants for further use.

Characterization of potassium solubilizing bacteria

Morphological characterization

All the selected isolates were examined for the colony morphology *viz.,* colour, cell shape, ability to form spores and Gram reaction as per the standard procedures.

Colony morphology

The colony characters *viz*., colour, shape and size were observed on agar medium (Gerhardt *et al.*, 1981).

Gram staining & Microscopic examination (Rangaswami, 1975)

Gram staining of the inoculants was carried out as per Hucker's modified method (Rangaswami, 1975). A drop of sterilized distilled water was taken on the middle of each clear slide. Then a loopful of bacterial suspension (young culture) was transferred to the sterilized drop of water and a very thin film was prepared on each slide by spreading uniformly. The film was fixed by passing it over the gentle flame for two or three times. The slides were flooded with crystal violet solution and allowed to stand for 30 sec and then washed thoroughly with gentle stream of tap water. The slides were then immersed in iodine solution for one minute and washed thoroughly with 95 per cent alcohol for 10 seconds Alcohol was drained off and washed thoroughly with gentle stream of tap water. The slides were then covered with safranin for one minute. The slides were washed with distilled water and air dried. The cellular morphology of inoculants was observed under microscope.

Test for Sporulation (Seldin *et al*., 1984)

Some bacteria are capable of changing into dormant structures that are metabolically inactive and do not grow or reproduce. Since these structures are formed inside the cells, they are called endospores. These are remarkably resistant to heat, radiation, chemicals and other agents that are typically lethal to the organisms. A single bacterium forms single spore by process called sporulation. Sporulation takes place either by depletion of essential nutrient or during unfavourable conditions.

The spore induction medium was dispensed in five ml quantities in test tubes and was sterilized. The medium was inoculated with 0.1 ml each of 48 h test culture and incubated at room temperature for a week's period and 0.1 ml of each culture broth was taken and smear was made in each slide. The film was dried over flame by gentle heating. The slide was then placed over a beaker and five per cent malachite green was added drop wise on the slide. Boiling of the malachite

green was avoided by adding more malachite green. The slide was taken out of the stream and washed gently with tap water. The preparation was stained with safranin solution for one minute and washed with gentle stream of tap water and placed under immersion lens with immersion oil.

Biochemical characterization

The biochemical characterization of the isolates was carried out as per the standard procedures as detailed below.

Catalase test (Blazevic and Ederer, 1975)

Nutrient agar slants were inoculated with test culture and were incubated for 24 h at 30 °C. The tubes were flooded with one ml of three per cent hydrogen peroxide after incubation. Production of gas bubbles was taken as positive for catalase activity.

Casein hydrolysis (Seeley and Vandemark, 1970)

The test organisms were streaked on petri plates containing skim milk agar and were incubated for one week at 30 °C. Clear zones around the colony were considered as positive for casein hydrolysis.

Starch hydrolysis (Eckford, 1927)

The test organisms were inoculated to starch agar plates and were incubated for 72 h at 30 ºC. After incubation the plates were flooded with Lugol's iodine solution and allowed to stand for 15-20 minutes. Clear zone around the colony was taken as positive for starch hydrolysis.

Urease test (James and Sherman, 1992)

The bacterial isolates were inoculated to five ml of urea broth containing phenol red as indicator. The tubes were incubated at 30 °C for 24 to 48 h. Formation of dark pink colour was taken as positive and no change in colour was taken as negative for urease activity.

Acid and gas production (Seeley and Vandemark, 1970)

The isolates were tested for acid and gas production by inoculating five ml of presterilized glucose broth medium in test tubes containing Durham's tube and bromocresol purple (15 ml l-1 of 0.04 % solution) as pH indicator. The tubes were incubated for seven days at 30 °C. The accumulation of gas in the Durham's tube was taken as positive for gas production and the change in colour of medium from purple to yellow was taken as positive for acid production.

Oxidase test (Cappuccino and Sherman, 1996)

The overnight culture of the test isolate was spotted on trypticase soya agar plates and the plates were incubated for 24 h at 28±2 °C. After incubation, two to three drops of tetramethyl phenylenediamine dihydrochloride were added to the surface of the growth of test organism. The colour change to maroon was taken as oxidase positive.

Gelatine liquefaction (Blazevic and Ederer, 1975)

The test organisms were inoculated to test tubes containing sterilized nutrient gelatin. The tubes were incubated first at 28±2 °C for 24 h and then incubated in refrigerator at 4 °C for 30 minutes. The tubes which remained liquefied were scored positive and those which solidified during refrigeration were scored negative for the test.

Hydrogen sulphide production (Cowan and Steel, 1970)

Five ml of sterile medium was inoculated with bacterial isolates and were incubated at room temperature for 48 h. Formation of black ring in the medium was taken as positive for H2S production.

Methyl red test (Seeley and Vandemark, 1981)

MR-VP tubes were inoculated with test culture and one uninoculated tube was kept as control. The tubes were incubated at 28±2 °C for 48 h. Five drops of methyl red were added to each tube after incubation and gently shaken. Development of red colour was taken as positive and development of yellow colour was taken as negative for methyl red test.

Voger Proskauer test (Seeley and Vandemark, 1981)

Test cultures were inoculated to the pre-sterilized tubes containing MR-VP broth. The tubes were incubated at 28±2 °C for 48 h. Ten drops of Barritt's reagent A were added after incubation and was gently shaken followed by addition of ten drops of Baritt's reagent B. Production of rose colour was taken as positive for VP test.

Denitrification test

Overnight grown cultures of the test organism were inoculated to the tubes containing Nitrate broth with inverted Durham's tube. The tubes were incubated at 25 °C for two weeks. Accumulation of gas in the inverted Durham's tube was taken as positive for denitrification test.

Citrate utilization test (Simmons, 1976)

The citrate utilization test was performed

by inoculating the microorganisms into an organic synthetic medium, Simmon's citrate agar, where sodium citrate was the only carbon and energy source. Bromothymol blue was added as an indicator. The Simmons citrate agar medium was prepared and poured in culture tubes equally and sterilized by autoclaving at 15lb pressure for 15 minutes to prepare slants. Inoculation was done by means of stab and streak method. After incubation at 28±2 °C for 48 h the slant cultures were observed for the growth and colouration of the medium.

Growth at 7 per cent NaCl

The test organisms were inoculated to nutrient broth tubes containing seven per cent sodium chloride and were incubated for 24 h at 28±2 °C. Development of turbidity was considered as positive for test.

Utilization of different carbon sources (Neyra *et al*., 1977)

The isolates were examined for their ability to utilize different carbon sources *viz.,* sucrose, mannitol and maltose. The carbon sources were added at the concentration of two per cent to the agar medium and 24 h old cultures were streaked on the surface of agar medium and incubated at 28±2 °C for 24 h. The extent of growth on the media containing different carbon sources was scored as no growth (-) and growth (+).

RESULTS AND DISCUSSION

Isolation of potassium solubilizing bacteria from rhizosphere soils of different crops

According to Altomare *et al.* (1999), a large population of mineral solubilizing microbes can be obtained from rhizosphere soil. Based on his point of view, the rhizosphere soils of different crops *viz.,* sorghum, maize, bajra, cotton, sunflower and groundnut were collected and used for the isolation of KSB. Out of the 75 isolates, 28 isolates were selected as potential KSB based on zone of solubilisation. Among the 28 isolates, 9 were from Lingasugur, 11 from Raichur and 8 from Deodurga taluk. These isolates were purified, identified and maintained for further study (Table.1).

Qualitative analysis for K solubilization of the isolates is presented in Table 2. All the isolates were examined for their ability to solubilize muscovite mica on Aleksandrov media

Table 1. Locations and rhizospheric soils of different crops used for isolation of Potassium solubilizing bacteria

S. No.	Place	Crop	Isolate code
LINGASUGUR (TALUK)			
1	Lingsugur	Sorghum	KSBL - 1
2	Lingsugur	Sorghum	KSBL - 2
3	Lingsugur	Maize	KSBL - 3
4	Lingsugur	Bajra	KSBL - 4
5	Hulkunti	Sunflower	KSBL - 5
6	Hulkunti	Sunflower	KSBL - 6
7	Hulkunti	Cotton	KSBL - 7
8	Hulkunti	Maize	KSBL - 8
9	Mudgal	Maize	KSBL - 9
10	Mudgal	Groundnut	KSBL - 10
11	Mudgal	Bajra	KSBL - 11
12	Mudgal	Sunflower	KSBL - 12
13	Mattur	Sorghum	KSBL - 13
14	Mattur	Maize	KSBL - 14
15	Mattur	Maize	KSBL - 15
16	Mattur	Maize	KSBL -16
17	Santekellur	Sunflower	KSBL - 17
18	Santekellur	Sunflower	KSBL - 18
19	Santekellur	Sorghum	KSBL - 19
20	Santekellur	Sorghum	KSBL - 20
21	Kalapur	Cotton	KSBL - 21
22	Kalapur	Bajra	KSBL - 22
23	Kalapur	Maize	KSBL - 23
24	Kalapur	Sunflower	KSBL - 24
25	Kalapur	Cotton	KSBL - 25
RAICHUR (TALUK)			
26	Raichur	Cotton	KSBR - 26
27	Raichur	Maize	KSBR - 27
28	Raichur	Maize	KSBR - 28
29	Raichur	Sunflower	KSBR -29
30	Eklaspur	Sunflower	KSBR - 30
31	Eklaspur	Sunflower	KSBR - 31
32	Eklaspur	Maize	KSBR - 32
33	Eklaspur	Maize	KSBR - 33
34	Askihal	Sorghum	KSBR - 34
35	Askihal	Bajra	KSBR - 35
36	Askihal	Bajra	KSBR - 36
37	Askihal	Cotton	KSBR - 37
38	Marchad	Sunflower	KSBR - 38
39	Marchad	Sorghum	KSBR - 39
40	Marchad	Sorghum	KSBR - 40
41	Marchad	Maize	KSBR - 41
42	Matmari	Maize	KSBR - 42
43	Matmari	Sunflower	KSBR - 43
44	Matmari	Bajra	KSBR - 44

45	Matmari	Cotton	KSBR - 45
46	Marchathal	Cotton	KSBR - 46
47	Marchathal	Cotton	KSBR - 47
48	Marchathal	Maize	KSBR - 48
49	Marchathal	Maize	KSBR - 49
50	Marchathal	Bajra	KSBR - 50
	DEODURGA (TALUK)		
51	Sultanpur	Maize	KSBD - 51
52	Sultanpur	Cotton	KSBD - 52
53	Sultanpur	Groundnut	KSBD - 53
54	Sultanpur	Maize	KSBD - 54
55	Gabbur	Sunflower	KSBD - 55
56	Gabbur	Sunflower	KSBD - 56
57	Gabbur	Sorghum	KSBD - 57
58	Gabbur	Maize	KSBD - 58
59	Masarkal	Maize	KSBD - 59
60	Masarkal	Sorghum	KSBD - 60
61	Masarkal	Cotton	KSBD - 61
62	Masarkal	Cotton	KSBD - 62
63	Arakera	Groundnut	KSBD - 63
64	Arakera	Groundnut	KSBD - 64
65	Arakera	Maize	KSBD - 65
66	Arakera	Sorghum	KSBD - 66
67	Navilur	Bajra	KSBD - 67
68	Navilur	Bajra	KSBD - 68
69	Navilur	Cotton	KSBD - 69
70	Navilur	Sunflower	KSBD - 70
71	Kotigudda	Sorghum	KSBD - 71
72	Kotigudda	Bajra	KSBD - 72
73	Kotigudda	Groundnut	KSBD - 73
74	Kotigudda	Sunflower	KSBD - 74
75	Kotigudda	Maize	KSBD - 75

Table 2. Solubilization zone formed by local isolates of potassium solubilizing bacteria

S. No.	Isolates	Zone of solubilization (cm)
1	KSBL - 2	0.60
2	KSBL - 5	1.20
3	KSBL - 8	0.40
4	KSBL - 9	1.00
5	KSBL - 12	0.50
6	KSBL - 13	0.60
7	KSBL - 18	0.80
8	KSBL - 23	0.30
9	KSBL - 25	0.50
10	KSBR - 28	0.40
11	KSBR - 30	0.60
12	KSBR - 31	1.00
13	KSBR - 32	1.00
14	KSBR - 35	0.60
15	KSBR - 38	0.70
16	KSBR - 41	1.30
17	KSBR - 42	0.50
18	KSBR - 44	0.30
19	KSBR - 46	0.90
20	KSBR - 50	0.40
21	KSBD - 51	0.50
22	KSBD - 55	0.70
23	KSBD - 58	1.40
24	KSBD - 63	0.30
25	KSBD - 64	0.60
26	KSBD - 67	1.20
27	KSBD - 72	0.50
28	KSBD - 74	1.00
29	Reference strain	1.30

supplemented with mica at one per cent. The diameter of zone of solubilization formed by the isolates ranged from 0.30 cm to 1.40 cm at 72 hours after incubation (HAI). Among the isolates KSBD-58 recorded maximum solubilization zone of 1.40 cm followed by KSBR-41 with the solubilization zone of 1.30 cm, the reference strain (*Frateuria aurantia*) also showed solubilization zone of 1.30 cm. The lowest solubilization zone of 0.30 cm was observed with isolates KSBL-23, KSBR-44 and KSBD-63.The results are in agreement with the findings of Prajapati and Modi (2012), where in, they isolated 5 efficient strains of KSB from soil samples of ceramic industries using mineral potassium as sole source of potassium. The results of present study strongly supported by the facts drawn by Hu *et al.* (2006), they isolated two phosphate and potassium solubilizing *Bacillus* sp. from the soils, in the modified medium containing phosphate and potassium minerals like kaolinite and potassium feldspar.

Morphological and biochemical characterisation of KSB isolates

All the 28 isolates were tested for colony morphology, Gram reaction, cell shape and spore formation. Eight isolates formed creamy white, slimy, small colonies; five isolates showed whitish, smooth, transparent colonies; four isolates were observed creamy white, smooth, small colonies; three isolates formed creamy white, smooth, large colonies; three isolates showed creamy white, opaque colonies; three isolates formed creamy white, small round spreading colonies and two isolates were observed creamy white smooth, small

Table 3. Morphological and biochemical characteristics of the potassium solubilizers

Isolate	Morphological characters Colony characters	Gram reaction & cell shape	Spore Formation	Biochemical test														Carbon sources			Probable genus
				1	2	3	4	5a	5b	6	7	8	9	10	11	12	13	14a	14b	14c	
KSBL-2	Creamy white, smooth, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBL-5	Creamy white, slimy, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBL-8	Creamy white, opaque	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBL-9	Creamy white, smooth, large	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBL-12	Creamy white smooth, smallelevated	- ve, rod	-	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Pseudomonas*
KSBL-13	Creamy white, slimy, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBL-18	Whitish, smooth , transparent	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBL-23	Creamy white, slimy, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBL-25	Creamy white, opaque	+ ve, rod	+	+	-	-	+	+	+	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBR-28	Creamy white, small round spreading	- ve, rod	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	+	*Pseudomonas*
KSBR-30	Whitish, smooth , transparent	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBR-31	Creamy white, smooth, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBR-32	Creamy white, slimy, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBR-35	Whitish, smooth , transparent	+ ve, rod	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	+	*Bacillus*
KSBR-38	Whitish, smooth , transparent	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBR-41	Creamy white, slimy, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBR-42	Creamy white, smooth, large	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBR-44	Creamy white, small round spreading	- ve, rod	-	+	-	-	+	+	+	-	+	-	-	-	-	-	-	+	-	+	*Pseudomonas*
KSBR-46	Creamy white, slimy, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBR-50	Creamy white smooth, large	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBD-51	Creamy white, smooth, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBD-55	Creamy white, small round spreading	- ve, rod	-	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Pseudomonas*
KSBD-58	Creamy white, slimy, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBD-63	Creamy white, slimy, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBD-64	Creamy white, opaque	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBD-67	Creamy white, smooth, small	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*
KSBD-72	Creamy white smooth, smallelevated	- ve, rod	-	+	-	-	+	+	-	+	-	+	-	-	-	+	-	+	-	+	*Pseudomonas*
KSBD-74	Whitish, smooth , transparent	+ ve, rod	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	+	*Bacillus*

1. Catalase test, 2. Casein hydrolysis, 3. Starch hydrolysis, 4. Urease test, 5a. Acid production, 5b. Gas production, 6. Oxidase test, 7. Gelatin liquification, 8. Methyl red test, 9. Voger proskauer text, 10. Denitrification test, 11. H_2S production, 12. Citrate utilization, 13. Growth at 7 % NaCl, 14a. Glucose, 14b. Maltose, 14c. Mannitol. + : Positive, - : Negativ.

elevated colonies. From Gram staining examination, five isolates *viz.,* KSBL-12, KSBR-28, KSBR-44, KSBD-55 and KSBD-72 were found to be Gram negative, whereas the remaining 23 isolates were observed as Gram positive with spore formation. All the isolates observed were rod in shape (Table 3). Based on the above mentioned results, 23 isolates were identified as *Bacillus* and five as *Pseudomonas* which was in accordance with findings of Prajapati and Modi (2012). Similar results were obtained by Avakyan *et al.* (1986) and Webley *et al.* (1960) wherein they observed morphological characterization revealing that all potassium solubilizing bacteria were gram positive short to long rods with spore production, but differed in their physiology and nutrition.

Biochemical characterization revealed all isolates positive for catalase, urease and acid production test, whereas 23 isolates showed positive for casein and starch hydrolysis test, four isolates observed positive for gas production test and 25 isolates showed positive for oxidase, gelatine liquification, methyl red and citrate utilization test. The ability of bacterial isolates to utilize different carbon sources (glucose, maltose and manitol) were also tested, wherein all isolates showed positive for glucose and manitol, but negative for maltose. All the isolates showed negative result for voger proskauer, dinitrification and H_2S production test (Table 3).

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