Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2020; 9(1): 1164-1168
Received: 28-11-2019
Accepted: 30-12-2019

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Development and evaluation of phosphate solubilising native yeast on sambar onion (*Allium cepa* var *aggregatum*)

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Abstract
In an attempt to isolate and evaluate native yeast on growth and yield of sambar onions. As many as four phosphate solubilising yeasts were isolated from the different fruits like pineapple sapota, banana and grape and all the yeast isolates were named as PSY - 1, PSY - 2, PSY - 3, and PSY - 4. Further, the yeast isolates were characterized based on morphological and biochemical characters as Streptomyces species. In *in vitro* screening experiments, the PSY-4 yeast isolate showed maximum zone of solubilization of phosphorous of 1.3 cm on sperber's media and the same isolates released the maximum of inorganic phosphorous of 7.80% in to the sperber's broth media after 10th day of incubation. Whereas, the other three isolates also solubilised the phosphorous but comparatively less than the PSY - 4 yeast isolate. The efficient isolate PSY - 4 along with varied levels and sources of phosphorous were evaluated on growth and yield of onion. With reference to number of leaves, chlorophyll content, fresh and dry weight of plant, and the treatments where 100% N and K + 100% P through rock phosphate + PSY - 4 showed the better results over the control. However, with reference plant NPK content of sambar onion, the same treatment showed the maximum plant NPK content of 160.00, 198.33 and 168.33 mg/plant respectively revealing the influence of PSY - 4 on growth and yield of sambar onion.

Keywords: Isolation, evaluation, phosphate solubilization, yeast, onion

Introduction
With the advent of modern technology, man has been using variety of inorganic chemicals, fertilizers, pesticides, fungicides *etc*., which led into many of the problems in the ecosystem. However, presently there is an emphasis to reduce the inorganic inputs which are used extensively in agriculture. Soil is a very complicated natural ecosystem that acts as a pool for all the plant nutrients that are in fixed and available form. These nutrients are fixed in the soil by chemical reactions ultimately influencing the non-availability of nutrients to the plants. On the other hand, soils are dynamic systems with multiple interactions between organic and inorganic soil components and the interactions in soil are important for the micro-organisms mobility and availability of mineral nutrients. Numerous microorganisms, especially those associated with roots having ability to increase the plant growth by solubilizing or releasing the unavailable mineral nutrients and also increase soil fertility (Ledin *et al*.1996) [14]. In ecosystem with low inputs and without any fertilization or soil amendments by humans, the nutrients available to plants come from atmospheric inputs and weathering of soil minerals (Christophe *et al.* 2006) [6]. The mechanism of weathering was mainly related to production of both organic and inorganic acids and complexing compounds as well as exchange uptake of elements among the minerals, soil solution and plants (Berthelin, 1988) [5].
Phosphorous (P) is one of the major essential macronutrient for plants and is applied to soil in the form of phosphate fertilizers. Phosphorous plays an important role in plants in many physiological activities such as cell division, photosynthesis, development of good root systems and utilization of carbohydrates. Phosphorous deficiency results in turning brown accompanied by small leaves, weak stem and slow development. Out of sixteen plant nutrients phosphorous is commonly deficient in most of the natural soils since it is fixed as insoluble Iron and Aluminium phosphates in acidic soil (McLean, 1976) [16]. As a result of phosphorous fixation some of the micronutrients are unavailable to the plants. Out of these micronutrients Iron (Fe^{2+}), Manganese (Mn^{+2}) Aluminium (Al^{+2}) are the major ones that form complexes with other nutrients and are unavailable to the plants which ultimately affect the yield.
Sambar Onion (*Allium cepa var. aggregatum)* is one of the bulb crops which are a good spicy vegetable crop which is used for preparation of almost all dishes. The pungency of onion is due to sulphur containing compound allicin, which gives characteristic aroma to the products,

prepared using onion. The microorganisms being integral part of soil play diverse role in organic onion production by converting unavailable form of nutrients into available form. On the other hand, many of the microorganisms play role in separation of many of the pests and diseases of onion. Among different microorganisms used the nitrogen fixers, the phosphorous, potassium solubilizers, and biocontrol agents like *Trichoderma, Pseudomonas fluorescens* are extensively used for increased yield of onion. However not many attempts were made to utilize the native yeast for healthy production of onion.

Varsha *et al.*, (2010) [22] isolated 25 phosphorous solubilizing yeasts from rhizosphere, non-rhizosphere and fruit samples of Bhvanagar district. Among 25 yeast isolates, 6 yeasts belonging to genus Saccharomyces, *Hansenula klockera, Rhodotorola and Debaryomyces* exhibit at highest phosphate solubilizing ability under *in vitro* condition.

Gerretsen (1984), proved the concept of using soil microorganisms to improve mobilization of poorly available forms of soil P has been applied. A number of phosphate solubilizing microorganism have been detected in soil and rhizosphere of plants which constitutes a heterogeneous group comprising bacteria (including Actinomycetes and Cynobacteria) and fungi scanty reports are available about P solubilizing yeast *viz.*, isolation of soil yeast by Taha *et al.* (1969) [21], *Schwanniomyces occidentaluis* from rhizosphere soil of cowpea by Bardiya and Gaur (1974) [3], *Rhodotorula sp.* from soil by Subba Rao (1982) [20] and *Yarowiali polytica* by Vassilev *et al.*(2001) [24].

Conceptual design is important in developing new technologies and also to utilize the plant growth promoting yeast for sustainable onion cultivation. The basic of conceptual design is simply to first convince a model and then to devise a strategy and method for achieving the reality. Moreover one should adopt a philosophical attitude in applying microbial technology in sambar onion production and also for soil health management. Based on the previous work undertaken by different researches and also in view of great need for developing novel yeast isolates for healthy sambar onion production the attempt was made to isolate and evaluate phosphate solubilizing yeast on sambar onion under green house conditions.

Materials and Methods

The present investigation was conducted in the Department of Agricultural Microbiology, College of Agriculture, Shivamogga. The details of materials and methodology followed during the course of investigation are highlighted herein.

Collection of fruit samples

A total of 4 different fruit samples (pineapple sapota, banana and grape) were collected from in and around of Shivamogga and these samples were packed in separate sterile biodegradable polythene bags and brought to laboratory for isolation of different yeasts.

Isolation of native yeast

Fresh fruit samples which were collected were initially surface sterilized with 0.1% mercuric chloride and the trace of mercuric chloride adsorb on to the surface of the fruit is removed by washing with sterile distilled water and further fruit samples were macerated using sterile pestle and mortar and the macerated sample were serially diluted and plated on yeast extract peptone dextrose agar.

Identification and characterization of yeast

The identification of each yeast isolate was made up to genus level based on morphological and biochemical characters as explained by Barnett *et al.*, (2000) [4].

Morphological, Microscopic and biochemical investigation

The colonies were observed on MA (malt extract) and MYGPA (Malt Yeast extract Glucose Peptone Agar medium). The isolates were also grown in MA and MYGPA broth for determination of their cultural characteristics (pellicle, sedimentation or ring formation, colony, elevation of colony, texture of colony and shape of colony). Under microscope, morphology (shape, size, budding, etc.) of each cell of each yeast were recorded and for carbon and nitrogen assimilation, the basal medium (YEPDA) of Barnett *et al.*, (2000) [4] were used.

In Vitro Screening of Native Yeast Isolate For Their Phosphate Solubilizing Ability

Agar plate method

All the yeast isolates were spotted on Sperber's media for analyzing the phosphate solubilizing potentiality of each isolates. Based on the zone of solubilization of phosphorus on the media the phosphate solubilizing potentiality of the phosphorus solubilizing yeast was interpreted (Gaur, 1990) [8].

3.6.2 Chemical method

Isolates of the phosphate solubilizing yeast (10 ml of the overnight culture were inoculated to 100 ml of Pikovskaya's broth in 250 ml flask with equal number of uninoculated controls. The flasks were incubated on a mechanical shaker at 28^0 C for 10 days. The amount of pi released in the broth in flasks was estimated at 10 days after inoculation. The broth cultures of yeast were centrifuged at 9000 rpm for 20 minutes in a centrifuge to separate the supernatant from the cell growth and insoluble phosphate. The available pi content in the supernatant/filtrate was estimated by phosphomolybdic blue color method (Jackson, 1973) [12].

Development and evaluation of yeast formulation and growth and yield of onion.

The efficient phosphate solubilizing yeast were identified and pure cultured on yeast extract peptone dextrose agar medium and the carrier (talc based) formulations was prepared for green house evaluation.

Preparation of carrier-based formulations of phosphate solubilizing yeast

An appropriate quantity of yeast inoculated to YEPDB medium and incubated for 5 days later the broth medium containing the phosphate solubilizing yeast were mixed thoroughly for uniform dispersal of the cells and the blended carrier material (talc) were sterilized and the phosphate solubilizing yeast were mixed in the ratio of 3:1. Further, the formulated powder was shade dried and passed through a 100 mesh size sieve repeatedly for homogeneous mixing and stored in sealed plastic bags for further experiments (Sireesha, 2000) [19].

Evaluation of efficient phosphate solubilizing yeast formulation on the growth and yield of sambar onion

The talc-based formulations were evaluated for its influence on plant growth under greenhouse condition using sambar onion test crop and the inoculations were made as per the treatment mentioned below.

T_1=Control (RDF)
T_2=100% N and K + 50% through Single Super Phosphate (SSP) + PSY - 4
T_3=100% N and K + 100% through Single Super Phosphate (SSP) + PSY - 4
T_4=100% N and K + 50% through Rock phosphate (RP) + PSY - 4
T_5 =100% N and K + 100% through Rock phosphate (RP) + PSY - 4

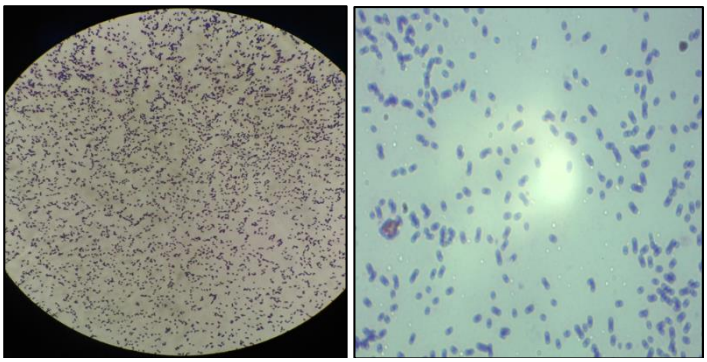
Note: The treatments were replicated in three replicates and further the observation on growth and yield parameters of sambar onion is recorded to know the treatment effects.

Results and Discussion

Isolation of phosphate solubilizing yeast

Four phosphate solubilizing yeast isolates were obtained from the fruit samples such as Pineapple, sapota, banana, and grapes (Table 1). The obtained isolates were named as PSY - 1, PSY - 2, PSY - 3 and PSY - 4 (Plate 1). The chance of isolating microbial isolates for solubilization of insoluble mineral nutrients is more in the soils of many crops (Alto mare, 1999) [2]. With this view four yeast isolates were isolated from the pineapple sapota, banana and grape fruit samples. It was interesting to note that ability of yeast isolates were able to grow and solubilize the medium containing fixed or insoluble form of nutrients (Sperber's media). The findings are in agreement with the results of Halverson *et al.* (1990) [10] who isolated aerobic yeast from different fruits, soils, marine environment soil and sewage samples.

Plate 1: Phosphate solubilizing yeast isolates isolated from different fruit samples



Under 10 X Under 40 X

Plate 2: Microscopic view of Phosphate solubilizing yeast isolate

Table 1: Collection details of phosphate solubilizing yeast

Sl. No.	Yeast isolates	Sample	Location
1.	PSY - 1	Pineapple	Local fruit market, Shivamogga
2.	PSY - 2	Sapota	Local fruit market, Shikaripura
3.	PSY - 3	Banana	Local fruit market, Bhadravathi
4.	PSY - 4	Grapes	Hopcoms, Shivamogga

Identification and characterization of yeast

In characterization study, the yeast isolates subjected to morphological, microscopic characteristics and attempts were also made to evaluate the different carbon source on growth and survivability of yeast isolates. Further, all the four yeasts, when inoculated to the media containing different carbon source and the yeast isolate PSY – 4 showed the capability of utilized all the carbon source tested indicating the diversity in using different carbon source in the nature. Based on the Morphological, Microscopic and carbon utilization ability results, all the yeasts were tentatively identified as *Saccharomyces sp*. (Table 2). The perusal of table 2 clearly indicates that all the isolates belongs to the genus Saccharomyces, similar results were also obtained by Leyval and Berthelin (1989) [15] who identified and characterized acid producing bacteria for phosphate solubilization.

Table 2: Identification characteristics of isolates of yeast

Sl. No.	Yeast isolates	Morphological characters					Carbon utilization potentiality										Probable genus
		Colony Color	Colony Surface on the MYGPA	Colony Margin	Colony Elevation	Cell shape under microscope	A	B	C	D	E	F	G	H	I	J	
1	PSY - 1	White	Rough	Entire	Slightly convex	Ellipsoid	+	+	-	+	+	-	+	+	-	-	*Saccharomyces sp.*
2	PSY - 2	Red	Smooth	Entire	Convex	Round/Ellipsoid	+	+	-	+	+	-	+	+	-	-	*Saccharomyces sp.*
3	PSY - 3	White	Rough	Undulating	Convex	Spherical	+	+	+	+	+	-	+	+	-	-	*Saccharomyces sp.*
4	PSY - 4	Red	Smooth	Entire	Convex	Round	+	+	+	+	+	+	+	+	+	+	*Saccharomyces sp.*

Note: A= D-Glucose test, B= D-Galactose, C= Sucrose, D= Maltose, E= Lactose, F= Starch, G= Glycerol, H= Manitol, I= Methanol, J= Ethanol, PG= Probable genus.

***In vitro* screening of phosphate solubilizing yeasts**

The results obtained on the zone of solubilization of phosphorus on Sperber's media and percentage of inorganic phosphate released by the phosphate solubilizing microbial isolates is furnished in Table 3. The highest zone of solubilization (1.3 cm) and maximum inorganic phosphate released was observed in PSY - 4 isolate at 10th day after the inoculation. However, the PSY-2 isolate also showed phosphate solubilization and pi release of 0.8 cm and 6.30%. Similarly, the PSY-1 and PSY-3 isolates performed less phosphate solubilization ability. Out of four yeast isolates screened the PSY-4 showed the highest phosphate solubilization ability hence PSY- 4 is selected for further evaluation studied (Plate 3). The results are in agreement with the findings of Gaind and Gaur, (1991) [7] isolated and screened *Bacillus megatherium, B.brevis, B. cerculianc, Bacillus subtilis* from rhizosphere of Oat and Arhar. Similarly, Murulikannan (1986) [17] isolated and screened silicate solubilizing bacteria from rice rhizosphere. Similarly Kannan and Raj (1998) [13] also screened 17 *Bacillus* species for their phosphorus and potassium solubilizing ability.

Plate 3: Phosphate solubilization potentiality of efficient yeast isolate (PSY - 4) on Sperber's Media

Table 3: *In vitro* screening of phosphate solubilizing yeasts

Sl. No.	Yeast isolates	Zone of solubilization (cm)	Amount of Pi release (%) at 10th day after incubation
1.	Control	0.00	3.30 (d)
2.	PSY-1	0.6	6.40 (bc)
3.	PSY-2	0.8	6.30 (c)
4.	PSY-3	0.5	6.60 (b)
5.	PSY-4	1.3	7.80 (a)
SEM±CD @ 1%			0.14 0.40

Development and evaluation of efficient phosphate solubilizing yeast - 4 on growth parameters of sambar onion.

Number of leaves and chlorophyll content

An evaluation of efficient PSY – 4 isolate with varied levels and different source of phosphorus was done to know the effect on number of leaves and chlorophyll content of the sambar onion. With reference to number of leaves the maximum number of leaves were observed in treatment 100% N and K + 100% through Rock phosphate + PSY – 4 (7, 16 and 18 at 30, 60 and harvest) followed by treatment 100% N and K + 100% through SSP + PSY – 4 *ie.*, 6, 14 and 16 at 30, 60 and harvest indicating the effect of PSY – 4 and varied levels and different source of phosphorus on number of leaves. On the other hand, the treatment number 5 showed significantly maximum chlorophyll content followed by treatment number 3. Whereas, the control treatment were having less chlorophyll content in the leaves. (Table 4). Similarly, the results are in agreement with the findings of Adesemoye *et al.,* (2008) [1] who evaluated different plant growth promoting *Psuedomonas, Bacillus and Saccharomyces species* on growth and yield of three vegetables like tomato, okra and amaranths. The results revealed that, at 60 days after planting the number of leaves were more in the consortial applications of *Psuedomonas, Bacillus and Saccharomyces*. The results do supported by Muthaura, *et al.,* 2010 [18] who evaluated consortia of effective microorganisms and growth and yield of pigweed which showed increase leaf number, leaf area and even chlorophyll content of the pigweed leaves, due to effective microorganisms.

Table 4: Effect of Phosphate solubilizing yeast on number of leaves and chlorophyll content.

Sl. No.	Tr. No.	Treatments	No. of leaves			Chlorophyll content (mg/g)
			30 days	60 days	At harvest	
1.	T_1	Control (RDF)	6	10	12	0.85(e)
2.	T_2	100% N and K + 50% P through SSP+PSY-4	5	12	13	1.30(d)
3.	T_3	100% N and K + 100% P through SSP+PSY-4	6	14	16	2.30(b)
4.	T_4	100% N and K + 50% P through rock phosphate+PSY-4	6	10	12	1.45(c)
5.	T_5	100% N and K +100% P through rock phosphate+PSY-4	7	16	18	3.20(a)
SEM±			0.04	0.03	0.46	0.08
CD at 1%			0.14	0.11	1.61	0.26

Effect of PSY- 4 on plant weight and plant nutrient status of Sambar onion

Significant variations among the treatment where observed in the fresh and dry weight of onion. Among treatments imposed, the treatment T5 recorded statistically high fresh and dry weight of 170 and 19 gram per plant followed by treatment 3 which showed 140 and 16 gram per plant. Whereas the control treatment showed the least fresh and dry weight of 96 and 9 gram per plant respectively. However, with reference plant NPK content of sambar onion. The same

treatment showed the maximum plant NPK content of 160.00, 198.33 and 168.33 mg/plant respectively revealing the influence of PSY - 4 on growth and yield of sambar onion with varied levels and different source of phosphorus Table 5. The findings are in line with the findings Ishque *et al.*, (2009) [11] who evaluated six different levels of nitrogen and phosphorus along with *Azospirillum* and phosphate solubilizing microbial isolates on growth, number of leaves, plant height and also nutrient status of the lettuce plant after harvest. Which was strongly supported by the results of Vasanthkumar, (2003) who concluded the maximum accumulation of residual nitrogen and phosphorous is more when the N-fixers and P solubilizers are used in the treatments. Scale up studies is required to commercialize the formulation of Phosphate Solubilizing Yeast for large scale application and also standard procedure for the quality control of PSY-4 formulations for effective usage in the organic farming practices.

Table 5: Influence of Phosphate solubilizing yeast on plant weight and NPK content of onion

Sl. No.	Tr. No.	Treatments	Weight of plant (g)		Nutrient (mg/plant)		
			Fresh weight (g)	Dry weight (g)	N	P	K
1.	T_1	Control (RDF)	$96^{(e)}$	$9^{(e)}$	$154.33^{(d)}$	$150.33^{(de)}$	$144.33^{(e)}$
2.	T_2	100% N and K + 50% P through SSP+PSY-4	$130^{(d)}$	$13^{(d)}$	$155.33^{(c)}$	$152.66^{(d)}$	$158.33^{(d)}$
3.	T_3	100% N and K + 100% P through SSP+PSY-4	$140^{(b)}$	$16^{(b)}$	$156.33^{(bc)}$	$190.00^{(b)}$	$166.67^{(b)}$
4.	T_4	100% N and K + 50% P through roc k phosphate+PSY-4	$138^{(bc)}$	$15^{(c)}$	$157.33^{(b)}$	$160.00^{(c)}$	$162.33^{(c)}$
5.	T_5	100% N and K +100% P through rock phosphate+PSY-4	$170^{(a)}$	$19^{(a)}$	$160.00^{(a)}$	$198.00^{(a)}$	$168.33^{(a)}$
SEM±			0.08	0.01	16.43	16.52	12.11
CD at 0.05%			0.21	0.04	2.04	2.61	2.26

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