

## AVERMECTIN PRODUCTION BY SOLID STATE FERMENTATION-A NOVEL APPROACH

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Received: 03 Jun 2017 Revised and Accepted: 31 Aug 2017

### ABSTRACT

**Objective:** The present study was carried out to evaluate SSF process for the production of avermectin by *Streptomyces avermitilis* NRRL 8165 using easily available grains, millets and some agricultural by-product.

**Methods:** Various substrates were screened for their ability to support avermectin production. Different parameters to maximize the yield of avermectin by *S. avermitilis* NRRL 8165 under SSF were optimized by conventional one factor at a time approach and parameters optimized earlier were adopted for the subsequent study.

**Results:** Sorghum seeds used as solid substrate supported maximum growth and total avermectin production (4.6 mg g<sup>-1</sup> dry substrate). The optimum values for maximum avermectin production were: moistening medium containing g l<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub> 1; MgSO<sub>4</sub>·7H<sub>2</sub>O 0.4, inoculum size 20 % (24 h old culture in yeast extract-malt extract dextrose medium) v/w of initial dry substrate, substrate particle size 0.5 to 4 mm, incubation temperature 28 °C, initial moisture level 105%, incubation period of 15 d, 8 % w/w sucrose and 5 % w/w soyameal. The avermectin yield with optimized fermentation condition was 5.8 mg g<sup>-1</sup> dry substrate which is 1.3 fold higher as compared to non-optimized condition.

**Conclusion:** Avermectin produced by *S. avermitilis* are widely used as an anthelmintic agent in the medical, veterinary and agricultural applications. In comparison with submerged fermentation, SSF can become an alternative cost-effective method for the production of avermectin. This report demonstrates the feasibility of employing agro-based substrate, that could reduce antibiotics production cost.

**Keywords:** Solid state fermentation, Avermectin, *Streptomyces avermitilis*, Sorghum, Optimization

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DOI: <http://dx.doi.org/10.22159/ijpps.2017v9i10.20470>

### INTRODUCTION

Avermectin produced by *Streptomyces avermitilis* are 16-membered macrocyclic polyketides antibiotics [1]. It has broad-spectrum anthelmintic activity against nematodes and arthropod parasites [2]. Recent testing revealed that avermectin also have potent antibacterial activity against various multidrug-resistant strains of *Mycobacterium tuberculosis* [3]. Presently, avermectin are exclusively produced by submerged fermentation (SmF) using mutants of *S. avermitilis*. Gao *et al.* [4] reported 5.1 g l<sup>-1</sup> avermectin B<sub>1a</sub> productions using a mutated organism *S. avermitilis* 14-12A at flask scale. These mutants are produced by various time and labour-intensive methods [5]. Chromosomal instability has also been reported in *S. avermitilis*, which is higher in mutant strains as compared to wild strain [6]. Novak *et al.* [7] reported instability in avermectin production, sporulation and pigmentation of *S. avermitilis* C-18/6 strain during subculturing. However, SmF has various disadvantages including a high volume of polluting effluent production, high volume and cost technology, high energy consumption, high risk of contamination, costly raw material, expensive bioreactor and complex downstream processing [8, 9].

Solid-state fermentation (SSF) has been developed as a microbial culture with solid substrates or impregnated inert support. SSF process is believed to mimic natural environment and encourage the microorganism to work at its best for the production of the product [9]. Compared with SmF, SSF has various advantages, including less water requirement, less energy, less production cost, less wastewater, simpler equipment, reduced volume of production media and reduced contamination risk [10]. SSF has very high potential for the production of secondary metabolites. Moreover, some secondary metabolites can only be produced under SSF conditions, like coniosetin, acremonidins A-E, pyrrocidienes A and B [11]. Several studies have been published recently on the production of secondary metabolites in SSF from *Streptomyces* like cephamycin C, tetracycline, oxytetracycline, actinorhodin and methylenomycin. SSF has been proved to be more

efficient for production of cephamycin C, tetracycline and oxytetracycline as compared to SmF [9].

The microbial metabolites biosynthesis greatly influence by physiological, nutritional and microbial parameters of fermentation process. To improve production of desire metabolite, optimization of these parameters were critically important [12]. Literature survey indicated that this organism has not been so far evaluated for the production of avermectin under SSF. The present study was carried out to evaluate SSF process for production of avermectin by *S. avermitilis* NRRL 8165 using easily available grains, millets and some agricultural byproduct.

### MATERIALS AND METHODS

#### Chemicals

An authentic sample of avermectin (abamectin, containing 80 % avermectin B<sub>1a</sub>) was procured from Baoding Jaihe Fermentation Co., Baoding, PRC. All other chemicals and solvents used were of AR grade except methanol (HPLC grade).

#### Microorganism

*Streptomyces avermitilis* NRRL 8165 a wild-type avermectin producer was used in the study and procured from northern regional research Laboratory, Peoria, Illinois, USA. It was stored in 20 % glycerol at -80 °C for long-term preservation. The working cultures of the microorganism were prepared by cultivation on yeast extract-malt extract glucose (YMG) agar and incubated at 28 °C until sporulation occurred (4-6 d) and slants were stored at 4 °C.

#### Solid state fermentation

All substrates like wheat bran, wheat rawa (wheat grains broken into small pieces of the size of 0.1-1 mm), seeds of sorghum, amaranth, pearl millet, barley, maize, potato (fresh) and sawdust were obtained locally. Except for wheat bran and wheat rawa, other substrates were

lightly crushed and passed through 40 and 10 mesh sieves. The fraction which passed through the 40 mesh sieve but retained by the 10 mesh sieve was collected and used as solid substrate (particle size 0.177 to 0.420 mm). All substrates were dried in an oven at 60 °C for 24 h. In 25×150 mm test tube, 2 g of solid substrate thoroughly mixed with 2 ml tap water (100 % initial moisture level) and autoclaved at 121 °C (15 psi) for 20 min. After sterilization, there was no free flowing water present in all the tubes. The inoculums were prepared by growing 1 ml spores suspension ( $1 \times 10^7$  spores/ml) in 25 ml YMG broth for 24 h under sterile condition. The sterilized initially 100 % moistened (v/w) substrate was inoculated with 0.4 ml of these

inoculums (20 % v/w of the initial dry substrate). The contents of the tubes were well mixed and incubated at 28 °C in incubation chamber humidified by keeping distilled water containing tray for various time periods. The substrate supporting the maximum production of avermectin was selected for further study.

#### Moistening media

Different moistening media, as reported in the literature for use with *Streptomyces* in SSF were tested (table 1). The avermectin production and biomass were analyzed in the fermented mass at 15<sup>th</sup> days of incubation.

**Table 1: Avermectin production and growth of *S. avermitilis* NRRL 8165 on sorghum with different moistening media in 15 d of incubation**

Moistening medium	Ingredients (g l <sup>-1</sup> )	Avermectin production <sup>a</sup>	Biomass <sup>b</sup>
A	KH <sub>2</sub> PO <sub>4</sub> 1; MgSO <sub>4</sub> ·7H <sub>2</sub> O 0.4; pH 7 [17]	5.21±0.035	141.12±3.21
B	KCl 0.02; MgCl <sub>2</sub> 0.02; pH 7 [30]	1.06±0.007	52.45±1.51
C	KH <sub>2</sub> PO <sub>4</sub> 5; NH <sub>4</sub> NO <sub>3</sub> 5, MgSO <sub>4</sub> ·7H <sub>2</sub> O 1, NaCl 1, CoCl <sub>2</sub> ·6H <sub>2</sub> O 0.001, MnSO <sub>4</sub> ·7H <sub>2</sub> O 0.0008, ZnSO <sub>4</sub> ·7H <sub>2</sub> O 0.0017, FeSO <sub>4</sub> ·7H <sub>2</sub> O 0.0025, pH 6.5 [18]	3.91±0.049	114.48±0.60
D	K <sub>2</sub> HPO <sub>4</sub> 5.3, NaHPO <sub>4</sub> 1.98, MgSO <sub>4</sub> ·7H <sub>2</sub> O 0.2, NaCl 0.2, CaCl <sub>2</sub> ·2H <sub>2</sub> O 0.02 [19]	2.82±0.280	101.71±2.26
E	MgSO <sub>4</sub> ·7H <sub>2</sub> O 0.01, KH <sub>2</sub> PO <sub>4</sub> 0.02; NH <sub>4</sub> NO <sub>3</sub> 0.05, NaCl 0.01, pH 7 [20]	4.30±0.120	125.15±0.98

<sup>a</sup>mg g<sup>-1</sup> dry substrate, <sup>b</sup>mg dry biomass g<sup>-1</sup> dry substrate, Values are the mean±standard error of 3 replicates

#### Effect of pH of the moistening medium

The effect of initial pH of the substrate on the production of avermectin was studied by varying the pH of moistening medium (Medium A) from pH 5.5-8.5. The pH was adjusted with 0.1N hydrochloric acid or 0.1N sodium hydroxide. To measure post-sterilization pH, 1 g of the sterilized substrate was stirred in 10 ml distilled water and pH was measured after settling the solid matter [13].

#### Optimization of the culture conditions for avermectin production

Different parameters to maximize the yield of avermectin by *S. avermitilis* NRRL 8165 under SSF were investigated. Parameter optimizes earlier was adopted during optimization of subsequent parameters. The effect of incubation temperature (24-28 °C), initial moisture content (60-120%), inoculums size (5-25 %) and substrate particle size on avermectin production was evaluated. The effect of additional carbon source (soluble starch, sucrose, maltose, glucose, molasses, lactose and fructose) all at 10 % w/w and additional nitrogen source (organic nitrogen source-soyameal, peanut meal, peptone, malt

extract and yeast extract at 5 % w/w, while inorganic nitrogen source—(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, KNO<sub>3</sub>, NaNO<sub>3</sub> and NH<sub>4</sub>NO<sub>3</sub> at 0.5 % w/w) were studied.

#### Extraction and analysis

For avermectin extraction, 0.5 g of the fermented substrate was extracted with methanol (5 ml) and the contents were agitated for 1 h at room temperature in a gyratory shaker at 150 rpm. The contents were centrifuged, and the pellet obtained was again mixed with another aliquot of 5 ml of methanol, kept on a gyratory shaker overnight and subsequently centrifuged. The supernatants were pooled, volume made-up to 10 ml and analyzed by HPLC method as described by Gao *et al.* [4] with some modifications. A C18 column (diameter 4.6×250 mm, length 284 mm, particle size 5 μ) was developed with methanol-water (85:15) at a flow rate of 0.5 ml min<sup>-1</sup>. The column temperature was set at 45 °C and products were monitored by UV detector at 245 nm. The quantity of total avermectin was calculated from the integration value at 245 nm using an authentic sample of avermectin as a standard [14].

**Table 2: Effect of initial pH of moistening media on selected parameters of sorghum, avermectin production and biomass accumulation of *S. avermitilis* NRRL 8165 in 15 d of incubation**

Moistening medium pH	Post-sterilization pH of substrate <sup>a</sup>	Avermectin production <sup>b</sup>	Biomass <sup>c</sup>	Reducing sugar <sup>d</sup>
5.5	6.86±0.12	4.90±0.08	131.2±4.20	2.47±0.040
6	6.87±0.15	4.88±0.05	134.2±3.80	1.92±0.113
6.5	6.89±0.09	4.78±0.07	129.5±2.11	1.69±0.125
7	6.89±0.08	4.96±0.040	132.3±4.36	1.61±0.040
7.5	6.88±0.15	4.92±0.035	134.8±4.11	1.68±0.025
8	7.00±0.12	5.01±0.045	129.9±5.30	1.34±0.030
8.5	7.57±0.10	4.93±0.069	129.8±2.69	1.58±0.049

<sup>a</sup>1 g of sterilized substrate was stirred in 10 ml distilled water and pH was measured after settling the solid matter, <sup>b</sup> mg g<sup>-1</sup> dry substrate, <sup>c</sup> mg dry biomass per gram of dry substrate, <sup>d</sup> reducing sugar released from sorghum after autoclaving in mg g<sup>-1</sup> wet substrate, Values are the mean±standard error of 3 replicates.

#### Biomass estimation

Biomass accumulated during the fermentation was estimated by the method reported by Kagiwal *et al.* [13]. A standard curve was prepared using N-acetyl glucosamine as standard (R<sup>2</sup>= 0.997) and was correlated to dry biomass of *S. avermitilis* NRRL 8165 grown in liquid culture (R<sup>2</sup>= 0.991). Biomass has been represented as mg dry biomass per gram of dry substrate (mg db g<sup>-1</sup> dsb).

#### Reducing sugar estimation of substrate

For analysis of reducing sugar released during autoclaving, 20 ml distilled water was added in 2 g of the autoclaved substrate and kept on a gyratory shaker at 180 rpm for an hour. The Clear solution was

separated from the substrate and the amount of reducing sugar released from substrate was determined by DNS method [15].

#### Statistical analysis

Statistical analyses were performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. Student's t-test was employed to investigate statistical differences and P<0.05 was considered significant. Unless otherwise indicated, all values were the means of three independent trials±standard deviations. No significant differences were observed between individual replicates. Pearson's correlation coefficient (r) and regression coefficient (R<sup>2</sup>) were also calculated.

## RESULTS

## Evaluation of various substrates for avermectin production

Different solid substrates viz. wheat bran, wheat rawa, sorghum, amaranth, pearl millet, barley, maize, potato and sawdust were screened for production of avermectin by *S. avermitilis* NRRL 8165. Five substrates were found to be capable of supporting the growth and production of avermectin. Fig. 1 shows the time course of avermectin production and biomass accumulation of these substrates. Sorghum was found to be the best substrate giving a maximum production of  $4.4 \pm 0.02$  mg g<sup>-1</sup>dsb (dry substrate basis) followed by wheat rawa, pearl millet and amaranth at 15 d. Rice gave the lowest production of avermectin ( $2.35 \pm 0.063$  mg g<sup>-1</sup>dsb) in same period of incubation. Further incubation did not show any significant increment in antibiotic production (fig. 1a). Sorghum also supported maximum biomass of  $133.63 \pm 3.73$  mg of db (dry biomass) g<sup>-1</sup> of dsb at 15 d. Amaranth, rice and pearl millet also showed growth of  $111.68 \pm 5.59$ ,  $83.71 \pm 3.62$ , and  $97.21 \pm 3.51$  mg of db g<sup>-1</sup> of dsb, respectively at 15 d. Wheat rawa produced maximum biomass at 20 d ( $99.66 \pm 13.8$  mg of db g<sup>-1</sup> of dsb) (fig. 1b), and further incubation after 20 d did not show increment in biomass (data not shown).

## Optimization of process parameters

Among the five different moistening media tested Medium A showed maximum production of avermectin ( $5.2$  mg g<sup>-1</sup>dsb) as well as biomass accumulation as compared to other moistening media (table 1). The effect of initial pH levels (5.5-8.5) of medium A on avermectin production during SSF was evaluated (table 2). Initial pH of moistening medium does not show a significant effect on biomass accumulation and avermectin production. To evaluate the effect of initial pH on sorghum, post-sterilization pH and reducing sugar content were measured. After sterilization, substrate pH and reducing sugar released during autoclaving did not show significant change along with different initial pH value of moistening medium (table 2). Also at the end of the fermentation pH of all tested variables did not change. Temperature affects microbial growth, spore formation, germination and microbial physiology, thus affecting product formation. Analysis of avermectin production revealed that maximum yield ( $5.40 \pm 0.156$  mg g<sup>-1</sup>dsb) is recorded at 28 °C (fig. 2). Reduced avermectin production was observed at higher or lower incubation temperatures. Fig. 2 represent the effect of inoculums level on avermectin production by *S. avermitilis* NRRL 8165 on sorghum. Progressive increases in product yield are observed with increase in inoculums from 5 to 20 % of the initial dry substrate and after that product yield slightly decrease. Maximum avermectin production of  $5.40 \pm 0.175$  mg g<sup>-1</sup> dsb was observed with 20 % inoculums level in 15 d.

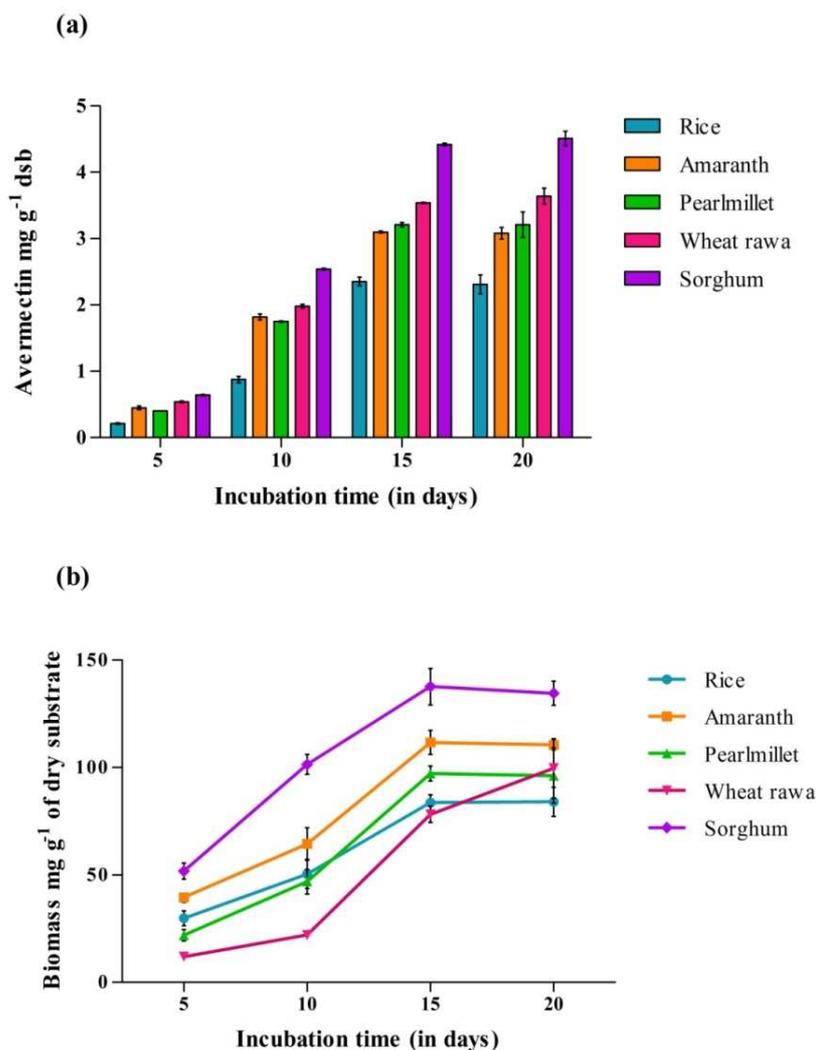
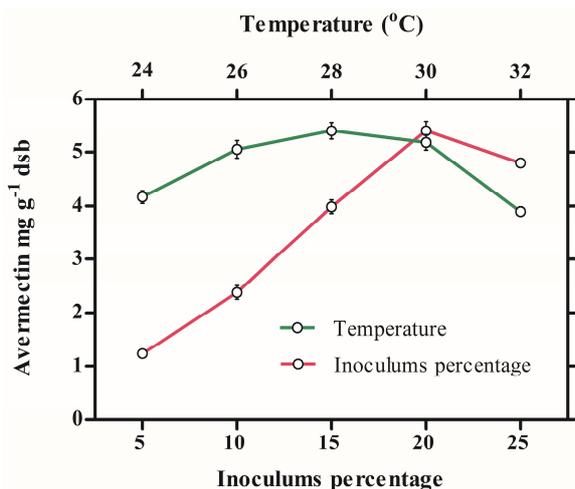


Fig. 1: Avermectin production and growth profile of *S. avermitilis* NRRL 8165 on solid substrates. (a) Represent avermectin production, (b) Represent biomass accumulation of *S. avermitilis* NRRL 8165 on different solid substrates. Initial moisture content 100 %, inoculums level 20 % v/w, incubation temperature 28 °C, incubation time 15 d. Values are the mean  $\pm$  standard error of 3 replicates



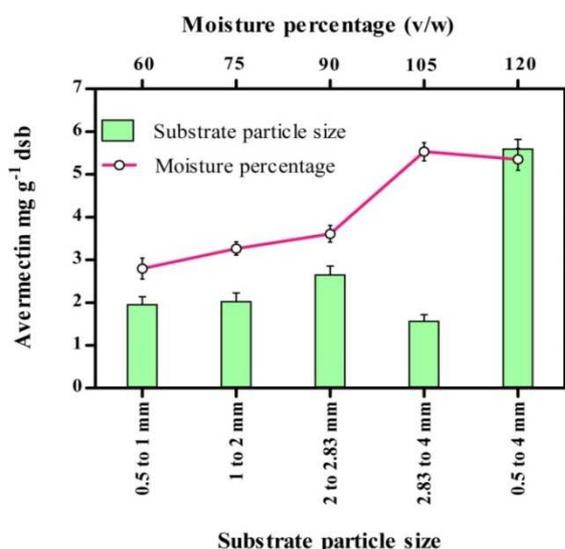
**Fig. 2: Avermectin production at selected inoculum level and temperature range by *S. avermitilis* NRRL 8165. Initial moisture content 100 %, incubation time 15 d. Values are the mean±standard error of 3 replicates**

The effect of initial moisture contents of the substrate (sorghum) on avermectin production is given in fig. 3. The result indicated that the maximum yield of avermectin ( $5.55 \pm 0.214$  mg g<sup>-1</sup> dsb) was obtained from 105 % initial moisture and a further increase in moisture levels reduced the avermectin content. After the fermentation, all the tubes with different initial moisture levels were showed relatively same final moisture content. At low moisture content, avermectin accumulation reduced because the water content was not sufficient enough for growth and metabolic activities. The experimental data revealed that avermectin production was affected by the particle size. Maximum avermectin production ( $5.61 \pm 0.210$  mg g<sup>-1</sup> dsb) was recorded with mixed size (0.5 to 4 mm) sorghum particles when initial moisture content was 105 % (fig. 3).

The impact of supplementation of external carbon sources on avermectin production was studied and the results have shown 20 % improvement in the avermectin production with sucrose and 12 % with maltose (both at 10 % w/w) (fig. 4). A marginal non significant improvement was observed with lactose. However, more than 35% reduced avermectin production was noticed with the supplementation of soluble starch suggesting that this carbon source could be a repressor of avermectin production in SSF. Glucose, fructose and molasses showed a slightly reduction in avermectin production. To determine the optimum sugar level, different concentrations of sucrose was supplemented in the range (6-14 % w/w). Maximum production (5.61 mg g<sup>-1</sup> dsb) was observed with 8 % sucrose concentration (fig. 4). Increase in the concentration of sucrose beyond this adversely affected avermectin production. The effect of different nitrogen sources on avermectin production by *S. avermitilis* NRRL 8165 was studied at optimized SSF environment (fig. 5). Results revealed that among selected nitrogen sources, soyameal at a given concentration (5 % w/w) showed highest antibiotic production, while all other nitrogen sources resulted in decrease production of antibiotic compare to control (fig. 5). Further evaluation of soyameal concentration showed a parabolic nature of production pattern indicating that this nitrogen source play a critical role on avermectin production (fig. 5).

## DISCUSSION

SSF has become an interesting alternative for the production of secondary metabolites since the metabolites are more concentrated, more stable and their downstream processing are easy as well as less costly [16]. There are several factors which affect the SSF processes, among these; selection of suitable substrate is crucial [9]. The solid substrates not only supply the nutrients for growth of microorganism, but also serve as an anchorage for the cell. Since no substrate was reported for production of avermectin using SSF.



**Fig. 3: Avermectin production at selected substrate particle size and moisture levels by *S. avermitilis* NRRL 8165. Inoculum level 20 % v/w, incubation temperature 28 °C, incubation time 15 d. Values are the mean±standard error of 3 replicates**

Different types of solid substrate were used for avermectin production in SSF. Among the tested substrates, five substrates (sorghum, amaranth, pearl millet, rice and wheat rawa) were supported avermectin production and growth of *S. avermitilis* (fig. 1). Sorghum supported maximum growth and avermectin production as compared to other substrates. Substrate-dependent variations in production of metabolites were also reported in the literature [17-22].

Production profile and growth curve of *S. avermitilis* NRRL 8165 on different substrates showed a positive correlation between growth and secondary metabolite production ( $r = 0.9941$ ;  $R^2 = 0.9883$ ). The possible reason for the reduced avermectin accumulation and growth on wheat rawa and rice could be attributed to the formation of aggregates, limiting the growth of microorganism by reducing surface area. Based on the above results, sorghum was found to be most suited amongst the tested substrates for growth and avermectin production by *S. avermitilis* NRRL 8165. In SSF, sorghum has been widely used for ethanol and organic acid production [8]. Sorghum contains 72.09 % carbohydrate, 10.62 % proteins, 6.7 % fibers, 3.46 % fatty acids, 2.53 % sugars, various minerals and vitamins [23]. It works as solid substrate and also contains all the components that could support growth of *S. avermitilis* NRRL 8165. However, this work apparently is the first report regarding use of sorghum for secondary metabolite production.

Medium A supported maximum biomass as well as avermectin production by this strain. Vastral and Neelagund [17] have reported this medium A for production of neomycin by *Streptomyces fradiae* NCIM 218. Many processes (ATP, DNA and RNA synthesis) occurring in the living system requires phosphorus [24]. Magnesium works as a cofactor of the enzymes involved in protein synthesis [25]. Ababutain *et al.* [26] reported that salt of magnesium and potassium are the most appropriate for growth and secondary metabolite production by *Streptomyces* species. Medium C and E containing NH<sub>4</sub>NO<sub>3</sub> led to relatively low levels of avermectin and biomass in the present case. Khaliq *et al.* [21] also reported the inhibitory effect of ammonium ion on tylosin production in SSF by *Streptomyces fradiae* NRRL 2702.

In most of the fermentation, the control of pH of the medium at optimum level is essential for achieving maximum product. Reports on secondary metabolite production under SSF showed a significant effect of initial pH of moistening medium on antibiotic production [13, 17, 19-22], but in this study pH of moistening medium did not show any effect in biomass and avermectin production (table 2). This may be attributed to the fact that sorghum has high buffering

capacity which resists change in pH as well as other tested parameters. Irrespective of type of fermentation, sizes of inoculum affect the formation of final product. As shown in fig. 2, optimum inoculums level was 20 % for the avermectin production. Lower and higher inoculums level than the optimum resulted in decreased

avermectin production. The Higher amount of inoculums may cause quick and too much biomass production thereby leading to fast nutrient depletion and ultimately reduce secondary metabolite synthesis. A low inoculums density leads to insufficient biomass and end product synthesis [21].

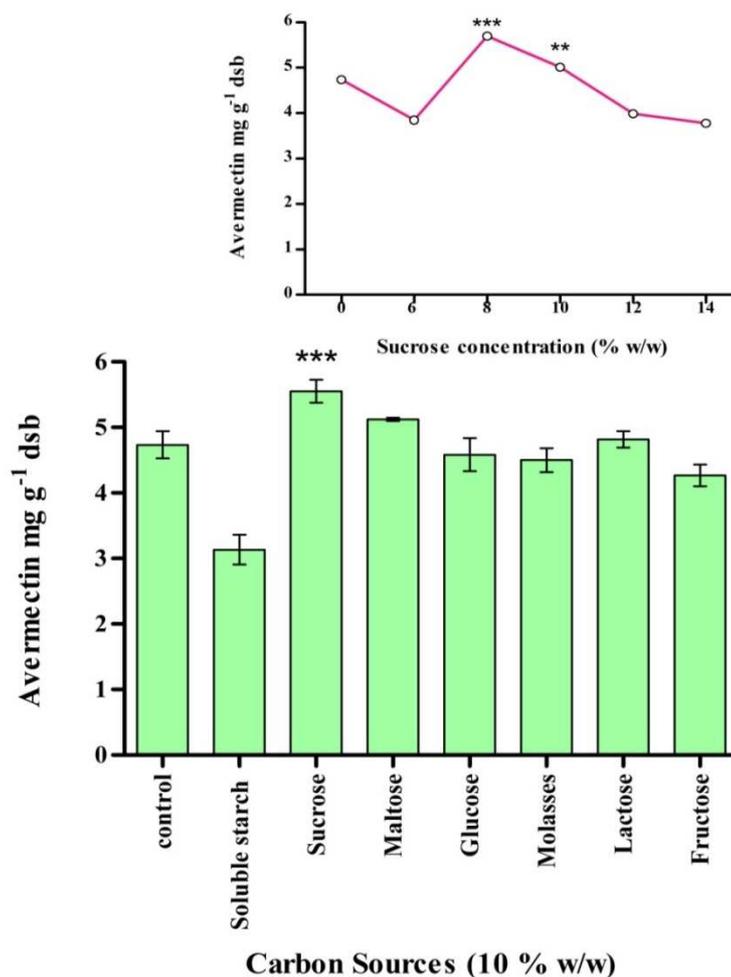


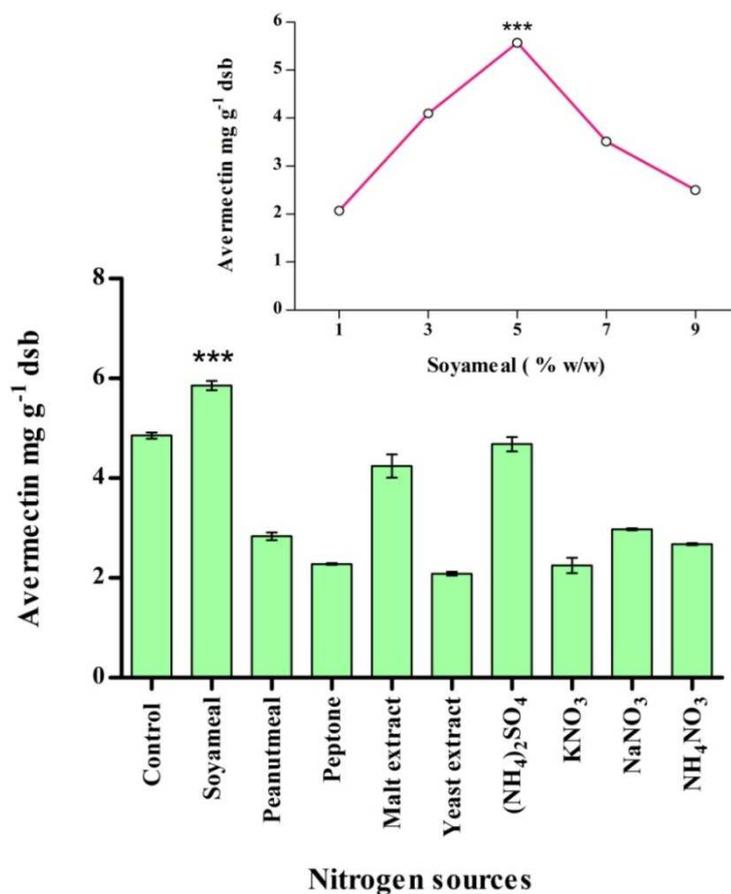
Fig. 4: Evaluation of carbon addition effect on avermectin production by *S. avermitilis* NRRL 8165 under SSF with sorghum as substrate (control: only sorghum). Inset effect of different concentration of sucrose. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.005$  variable vs control (student's t test)

In case of SSF, the Moisture level is one of the imported factor that affects growth and production of desire product [27]. The highest avermectin production was obtained at 105 % initial moisture contents (fig. 3). Low moisture decreased the availability of nutrients thus lowering the growth and finally reduces the production of secondary metabolites [28]. Under higher moisture level substrate porosity decreased which reduced mass transfer [29]. Similar observations were noticed in the literature [20, 22, 30] during SSF studies carried out for other metabolites.

Substrate particle size is a crucial factor in SSF process. Small particle sizes provide larger surface area for growth but decrease interparticle porosity. Larger particle size decrease surface area, limit nutrient transfer and increase interparticle space with suitable respiration and aeration characteristics. The particles sizes (0.5-4 mm) of the substrate were found to be optimal size of the substrate for maximum avermectin production (fig. 3). Data suggest that selection of substrate particle size is one of the essential requirements for production optimization in SSF [17, 22].

In solid substrate, some of the important nutrients necessary for growth and secondary metabolite synthesis for microorganism may also be present at sub-optimal level. Hence, the addition of other

nutrients may improve product formation in SSF processes. *S. avermitilis* NRRL 8165 can grow and produce avermectin on sorghum, but the organism may needed additional carbon source for maximum secondary metabolite synthesis. Different carbon sources were supplemented to solid substrate at 10 % (w/w). Results showed disaccharides supported, while monosaccharide's decreased avermectin production (fig. 4). Readily available carbon sources like glucose, fructose and molasses were reported to work as a repressor for enzymes which are involved in the synthesis of secondary metabolites [21]. Asagbra *et al.* [31] also reported the stimulatory effect of disaccharides in secondary metabolism. In SSF environment organism suffer from osmotic stress and synthesized solutes responsible for osmoregulation called compatible solutes. Sucrose is used as one of the compounds to reduce osmotic stress. Elibol [32] reported sucrose might also act as an enzyme system inducer responsible for the synthesis of a polyketide antibiotic actinorhodin produced by *Streptomyces coelicular* A3 (2). Yang and ling [33] also reported stimulatory effect of sucrose on tetracycline production with sweet potato residue by *Streptomyces viridiciens* ATCC 11989 under SSF. A similar observation was also reported in pikromycin production by *Streptomyces venezuelae* ATCC 15439. An optimum concentration of sucrose (139 g l<sup>-1</sup>) was required for pikromycin production [34].



**Fig. 5: Screening of different nitrogen sources avermectin production by *S. avermitilis* NRRL 8165 on sorghum. Inset effect of different concentration of soyameal. \* P<0.05; \*\* P<0.01; \*\*\*P<0.005 variable vs control (student's t test), organic nitrogen source - soya meal, peanut meal, peptone, malt extract and yeast extract at 5 % w/w, while inorganic nitrogen source - (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, KNO<sub>3</sub>, NaNO<sub>3</sub> and NH<sub>4</sub>NO<sub>3</sub> at 0.5 % w/w**

Soyameal is complex, cheap and commercially available nitrogen sources and has advantage of slow breakdown during the fermentation. Addition of soyameal (at 5 % w/w) has a positive effect on avermectin biosynthesis under SSF (fig. 4). When different concentration of soyameal (1-9 % w/w) was added, it showed a parabolic graph of avermectin production (fig. 4). Li *et al.* [35] reported soyameal is the best nitrogen source for streptolydigin A production by *Streptomyces lydicus* AS 4.2501. Bhavna *et al.* reported that maximum growth and antimicrobial compound production by *Streptomyces carpaticus* MTCC 11062 required an optimum concentration of soyameal [36]. Mahalaxmi *et al.* [22] also reported a critical role of soyameal in rifamycin B production by *Amycolatopsis* sp RSP 3 under SSF. In the present study with the optimum fermentation conditions, maximum avermectin production (5.8 mg g<sup>-1</sup>dsb) was recorded in 15 d of incubation.

#### CONCLUSION

Present work represents the first report of avermectin production by *S. avermitilis* in SSF condition. Maximum avermectin production was recorded with sorghum as a substrate. Initial pH of moistening medium does not affect the biomass and avermectin production on sorghum in the tested range. The identified and optimized process parameters include 105 % initial moisture content, 20 % (v/w) inoculum, incubation at 28 °C, incubation time 15 d, 8 % sucrose (w/w) and 5 % (w/w) soyameal. Under the optimal condition, the avermectin production of 5.8 mg g<sup>-1</sup>dsb has achieved an approximated 1.3 fold improvement over initial yield (4.42 mg g<sup>-1</sup>dsb) with non-optimized conditions. It was reported that under SmF condition *S. avermitilis* NRRL 8165 (ATCC 31267) produce 0.175 mg ml<sup>-1</sup> avermectin [37], while in optimized SSF condition this strain produces 5.8 mg g<sup>-1</sup>dsb avermectin. In future SSF process for

avermectin can become an alternative to classical submerged fermentation.

#### ACKNOWLEDGEMENT

Shadab Khan thanks, Council of Scientific and Industrial Research (CSIR), India for Senior Research Fellowship [09/301/(0124)/2012/EMR-I].

#### AUTHORS CONTRIBUTION

All authors contributed equally

#### CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest in the publication

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#### How to cite this article

- Shadab Khan, Pushpendra Awadhiya, Shridhar Patil, Tushar Banerjee. Avermectin production by solid state fermentation-a novel approach. *Int J Pharm Pharm Sci* 2017;9(10):55-61.