ISSN-2277-6079

Short article

# ENDOPHYTIC FUNGI: ARE THEY POTENTIAL CANDIDATES FOR THE PRODUCTION OF POLYUNSATURATED FATTY ACIDS?

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**Abstract**

One hundred and fifty endophytic fungi were isolated from different plant samples. Their total lipid and fatty acid profile were analysed. Our results show that the total lipid content in 50% of the isolated endophytic fungi ranged between10-14% and in the remaining 50% ranged between 7-9%. None of the endophytic fungi tested were found to be oleaginous in nature (accumulating more than 20% lipid). The endophytic fungi produced saturated fatty acids i.e palmitic acid, stearic acid, and monounsaturated- oleic acid in the range of 13-23%, linoleic acid from 40-50%, and alpha-linolenic acid- 2-14%. Few endophytic fungi accumulated arachidonic acid in a very low concentration i.e. 0.1-0.3%. The results of our study suggest that, endophytic fungi are capable of producing the precursors of PUFAs i.e. linoleic acid and alpha-linolenic acid but not the pharmaceutically important PUFA's as such. Our work also revealed that, there is not much difference in fatty acid profile of all the endophytic fungi isolated by us, irrespective of the differences in the living conditions (such as nutritional and environmental parameters) of the plants from which they were isolated.

**Key words:** Alpha- linolenic acid, Endophytic fungi, Linoleic acid, Polyunsaturated fatty acids.

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## Introduction

Polyunsaturated fatty acids are the important components of human nutrition. These fatty acids are characterized by at least two carbon-carbon double bonds in a hydrophobic hydrocarbon chain. They cannot be synthesized by our body and hence have to be obtained through diet and therefore they are referred as essential fatty acids (EFA).There are two families of EFA namely ω -3series, derived from alpha-linolenic acid (ALA 18:3) and ω -6 series, from linoleic acid (LA). The ω -6 series are gamma-linolenic acid (GLA, 18:3 *n*−6), the 20 carbon dihomo-gamma-linolenic acid (DGLA, 20:3 n-6) and arachidonic acid (ARA, 20:4 *n*−6). Similarly ALA converts into 20 carbon eicosapentaenoic acid (EPA, 20:5 *n*−3) and 22 carbon docosahexaenoic acid (DHA, 22:6 *n*−3) [1]. In the early 20th century, Burr and Burr first recognized the importance of EFA in public health and disease [2]. Polyunsaturated fatty acids regulate a wide range of functions in human body including blood pressure [3], vasoconstriction [4], vasodilation [5], vision [6] and normal development, maintenance and functioning of the brain [7]. Furthermore, PUFA's play an important role in regulating inflammatory responses by modulating arachidonic acid synthesis by producing mediators termed as eicosanoids [8].

Endophytic fungi are a group of fungi living inside the host plant tissues for all or part of their life cycle, cause no apparent infections and are known to occur ubiquitously in plants [9]. Endophytic fungi have the ability to adapt to its host and external environment during stress and unfavorable conditions. They are considered as treasure house of secondary metabolites when it is grown *in vitro* under controlled conditions [10]. Thus endophytic fungi have been recognized as an alternative source for various secondary metabolites in nutraceutical and pharmaceutical industry. They are also capable of producing primary metabolites such as gibberellins [11], enzymes like lipase [12], laccase [13] , amylase [14], pigment [15], chitoson [16], biodiesel [17] etc. A very few evidences are available on lipid production in general and PUFA in particular. The main aim of the present work was to screen endophytic fungi isolated from different plant sources to ascertain its ability to produce pharmaceutically important polyunsaturated fattyacids.

## Materials and methods

### Microorganisms and culture conditions

One hundred and twenty eight endophytic fungi were isolated from different plants like, *Portulaca oleracea, Helianthus annuus, Eucalyptus globules, Cymbopogon flexuosus, Cicer arietinum, Vigna unguiculata, Arachis hypogaea, Ricinus communis, Plectranthus amboinicus* and *Glycine max*. Twelve endophytic fungal cultures were obtained from Centre for Advanced Studies (CAS) in Botany, Madras University, Chennai, India and ten endophytic fungal cultures were obtained from Dept of Applied Botany, Mysore University, Mysore, India. Surface sterilization was done by following the method of Suryanarayanan et al [18]. All leaf and stem samples were cut into 5-7mm pieces washed twice in distilled water, surface sterilized by immersing for 1 minute in 70% (v/v) ethanol, 4 minutes in sodium hypochlorite (3% (v/v) available chlorine) and 30 seconds in 70% (v/v) ethanol and then washed three times in sterile distilled water for 1 minute each time. After surface sterilization, it was aseptically transferred to petriplates containing Potato Dextrose Agar (PDA), pH 6.8, containing (g $l^{-1}$): potato, 200; dextrose, 20; agar, 15), autoclaved for 15 minutes at 121ºC and then aseptically supplemented with 100 mg/ml of streptomycin to suppress bacterial growth. Aliquot from the third wash was plated onto PDA to check the effect of surface sterilization.

**Growth conditions of fungi**

The endophytic fungi was maintained on PDA and was grown in a medium (nitrogen limiting) of the following composition (g $l^{-1}$) $KH_2P0_4$, 7.0; $NH_2HP0_4$, 2.0; $MgS0_4.7H_20$, 1.5; yeast extract, 1.0; $Cacl_2.2H_20$, 0.1; $FeCl_2.4H_20$, 0.008; $ZnS0_4.7H_20$, 0.0001; di-ammonium tartarate, 1.5;the glucose concentration was 10g $l^{-1}$ and the pH was adjusted to 5.5 with 0.1N NaOH. The cultures were grown in 50 ml medium in 250 ml Erlenmeyer flasks for 4 days at 28°C on a rotary shaker at 120 rpm. The biomass after growth was harvested by filtration, washed thoroughly with distilled water, lyophilized, weighed and used for further analysis [19].

**Lipid extraction and fatty acid analysis**

The dried fungal mass was made into fine powder using mortar and pestle. Extraction of total lipid was done by following the method of Folch et al [20]. Known amount of powdered mycelial mass was mixed with 100 ml of chloroform: methanol (2:1 v/v) and the lipid was extracted using a separating funnel. The lower fraction was collected in a beaker and allowed to evaporate till dryness. The residue containing lipid was dissolved in diethyl ether and later was transferred to pre-weighed sterilized vials, allowed to evaporate till dryness and weight of the vials were again recorded. The fatty acids of total lipids were analyzed as their methyl esters by gas chromatography according to Certík et al [21].

The gas chromatograph (GC-6890 N, Agilent Technologies) was equipped with a capillary column DB-23 (60m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies) and a FID detector (constant flow, hydrogen 35 ml/min, air 350 ml/min, 250°C). Analyses were carried out under a temperature gradient (130°C for 1 min;130–170°C at program rate 6.5°C/min; 170–215°C at program rate 2.7°C/min; 215°C for 7 min; 220–240°C at program rate 2°C/min; 240°C for 2 min) with hydrogen as a carrier gas (flow 2.1 ml/min, velocity 49 cm/s, pressure 174 kPa) and a split ratio of 1/50 (inlets: heater 230°C, total hydrogen flow 114 ml/min, pressure 174 kPa). The fatty acid methyl ester peaks were identified by authentic standards for a C4–C24 fatty acid methyl ester mixture (Supelco, USA) and quantified by an internal standard of heptadecanoic acid (C17:0, Supelco, USA). The fatty acid concentration was evaluated with ChemStation software B0103 (Agilent Technologies, USA). All values were means of triplicate determinations.

**Results**

The total lipid content in 50% of endophytic fungi ranged between 10-14% and in the remaining 50% ranged between 7-9% (Table 1). The total lipid content of endophytic fungi isolated from *Cicer arietinum, Plectranthus amboinicus, Vigna unguiculata, Ricinus communis, Helianthus annuus* and *Eucalyptus globules* ranged between 6-8% and 9-14% from *Cymbopogon flexuosus, Arachis hypogaeas, Portulaca olerace and Glycine max*. The highest total lipid content among the endophytic fungal isolates was 14% isolated from *Portulaca oleracea* followed by endophytic fungi isolated from *Glycine max* (13%). These two plants are reported to have lipids rich in ALA and LA (28-39% and 50-57%) respectively. Perhaps this could be one of the reasons for the highest total lipid content (i. e 14%) in fungi isolated from these plants, compared to endophytic fungi isolated from other plants , although they were grown *in vitro* under controlled conditions. However, we do not have any physiological evidence to show that the plant lipid content influences the endophytes in lipid production.

A very few studies are available on fatty acid composition of endophytic fungi [22]. Our results suggests that the main fatty acids produced by the endophytic fungus is palmitic acid,

stearic acid, oleic acid in the range of 13-23% (Fig 1) and this data correlates with other studies [23, 24]. The linoleic acid content ranged between 9-50% and few endophytic fungi (JSK1, JSK3, JSK4, JSK5, JSK7, JSK13, JSK19, JSK20) produced linoleic acid at high levels ranging from 40-50%, similar to those found in soybean oil. Similarly the endophytic fungi (JSK6 and JSK8) isolated from *Portulaca oleracea* produced total lipid content ranging between 12-14% and 13-14% (Table 1) alpha-linolenic acid. Endophytic fungi isolated from *Glycine max* (JSK10 and JSK11) produced a small amount of arachidonic acid in the range of 0.1 and 0.3% respectively.

**Fig.1 Relative percentage of fatty acid composition of endophytic fungi isolated from different plant sources. C14:0 (Myristic acid); C16:0 (Palmitic acid); C18:0 (Stearic acid); C18:1(oleic acid); C18:2 (linoleic acid); C20:4 (Arachidonic acid); C22:0 (Docosanoic acid); C24:0 (Tetracosanoic acid).**

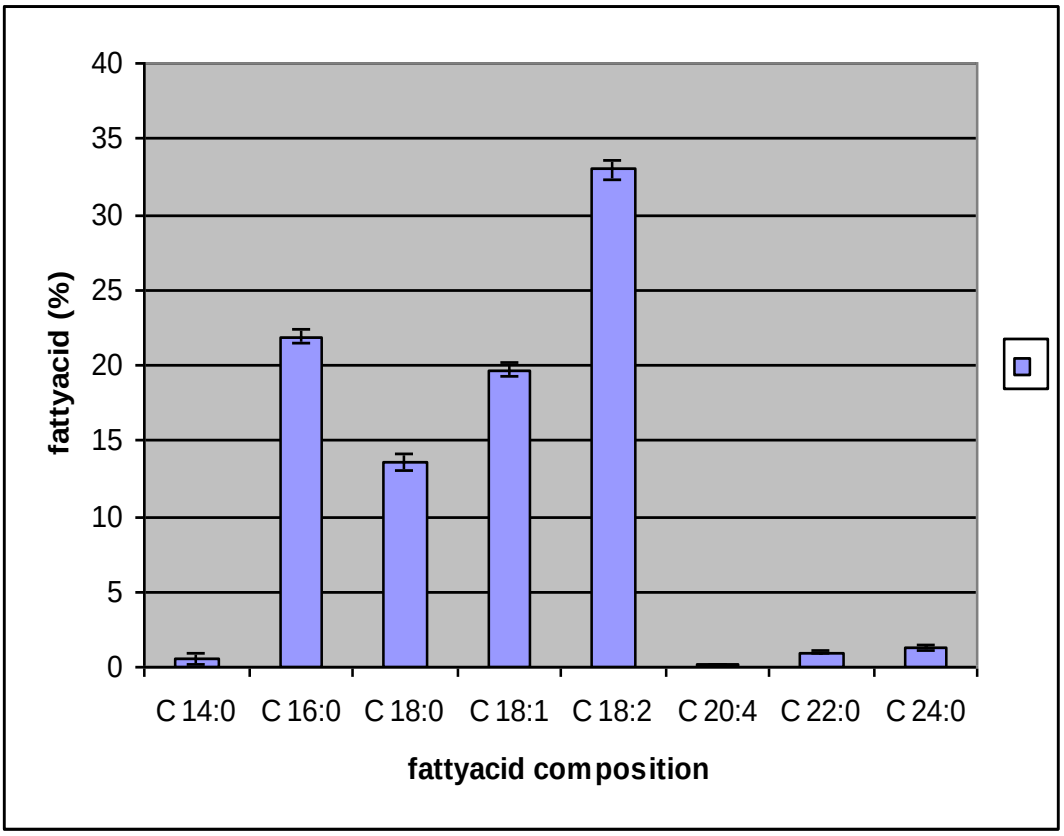

**Table 1: DBM: Dry biomass; TL: Total lipid; LA: Linoleic acid; ALA: Alpha- linolenic acid; ARA: Arachidonic acid; yield of some endophytic fungi isolated from plant sources**

| STRAIN NUMBER | DBM(g/l) | TL (%) | LA (%) | ALA (%) | ARA (%) |
|---|---|---|---|---|---|
| **JSK1** | 3.34±0.9 | 7 | 46.14 ±1.45 | 4.03 ±0.58 | - |
| **JSK2** | 0.53±0.1 | 7 | 33.85 ±0.68 | 2.76 ±0.65 | - |
| **JSK3** | 3.34±0.8 | 11 | 48.68 ±1.09 | 3.29 ±0.10 | - |
| **JSK4** | 2.53±0.7 | 13 | 50.12 ±0.48 | 1.67 ±0.58 | - |
| **JSK5** | 3.40±0.1 | 7 | 44.83 ± 1.27 | 7.65 ±1.18 | - |
| **JSK6** | 2.78±0.6 | 14 | 10.16 ±1.71 | 13.76 ±0.34 | - |
| **JSK7** | 2.88±0.4 | 6 | 40.50 ±0.65 | 0.66 ±0.26 | - |
| **JSK8** | 3.03±0.7 | 12 | 9.06 ±1.59 | 14.24 ±0.79 | - |
| **JSK9** | 2.50±0.3 | 8 | 33.86 ±1.34 | 4.03 ±0.21 | - |
| **JSK10** | 2.41±0.4 | 9 | 30.22 ±0.54 | 3.72 ±0.26 | - |
| **JSK11** | 3.21±0.9 | 10 | 23.09± 0.58 | 2.90±0.92 | 0.11±0.1 |
| **JSK12** | 2.64±0.7 | 8 | 25.02 ±0.73 | 6.05 ±1.28 | 0.20±0.1 |
| **JSK13** | 3.0±0.84 | 8 | 40.35±0.24 | 4.25±1.21 | 0.155 |
| **JSK14** | 4.0±0.58 | 7 | 35.62±0.15 | 6.84±0.65 | - |
| **JSK15** | 1.9±0.05 | 10 | 36.14±0.84 | 2.64±0.54 | 0.17 |
| **JSK16** | 3.65±0.58 | 9 | 18.67±0.68 | 4.58±0.37 | - |
| **JSK17** | 2.54±0.39 | 6 | 25.29±0.51 | 6.71±0.24 | 0.3 |
| **JSK18** | 3.84±0.18 | 8 | 20.58±0.35 | 5.61±0.67 | - |
| **JSK19** | 3.41±0.84 | 7 | 41.66±0.48 | 8.34±0.16 | - |
| **JSK20** | 3.72±0.47 | 10 | 45.74±0.22 | 3.57±0.18 | - |

## Discussion

Not many reports are available on the analysis for fatty acid composition of lipids in endophytic fungi. Our present report is one among the few of its kind, where a large number of endophytic fungi (one hundred and fifty) were isolated in order to ascertain the ability of PUFA accumulation in endophytic fungi. We also report for the first time the accumulation of ARA although in a very small concentration of 0.1 and 0.3% in a few endophytic fungi. In conclusion, our findings in the present study reveals that the endophytic fungi are a poor candidate for the production of therapeutically important PUFAs against the soil fungi which have been reported for long as the best source of polyunsaturated fatty acid.

## Acknowledgements

The author's acknowledge University Grant Council (UGC), India for funding research project (F.No.34-243/2008) entitled "Lipid profile of endophytic fungi: Identification of suitable strain for the production of commercially important omega fatty acids (EPA & DHA)".

## Conflict of interest

The authors declare that they have no conflict of interest.

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