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Relative growth and sporulation of *Nomuraea rileyi* (Farlow) Samson isolates on media with various carbon sources

T. SONAI RAJAN and N. MUTHUKRISHNAN
Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai 625 104,Tamil Nadu, India.
E-mail: snraja_insect@yahoo.co.in

ABSTRACT: **Evaluation of *Nomuraea rileyi* isolates collected from Project Directorate of Biological Control (PDBC) Bangalore, Directorate of Oilseeds Research (DOR) Hyderabad and a local isolate on media with different carbon sources showed that potato maltose agar was significantly superior over all other media for maximum biomass, mycelial growth and conidial production. Among the three isolates studied maximum radial growth, biomass and spore production were observed in PDBC isolates both in solid as well as liquid media.**

KEY WORDS: Biomass, carbon sources, *Nomuraea rileyi*, radial growth, spore production.

INTRODUCTION

Success of any microbial control programme depends on production of sufficient quantity of inoculum for field application. Most of the entomopathogenic fungi are facultative pathogens and can be mass produced in synthetic, semi-synthetic or natural media containing suitable nutrient sources. Selection of strains of fungi having high virulence, good growth and sporulation is considered important in mass culturing. Hence the present study was taken up to test six different carbon sources for their suitability to growth and development of *N. rielyi* isolates.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF *N. RILEYI*

Sources

Pure cultures of *N. rileyi* were obtained from Project Directorate of Biological Control (PDBC), Bangalore and Directorate of Oilseeds Research (DOR), Hyderabad. Surveys were also conducted in cotton, tomato, castor and pulses cropping areas of Coimbatore District (Thondamuthur) and *N. rileyi* infected cadavers were collected and maintained as local isolates. The three isolates of *N. rileyi* (PDBC, DOR and one local isolate from Coimbatore) were maintained at the Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai. The fungal isolates maintained in the standard mycological medium (Sabouraud's Maltose agar yeast extract medium) and incubated for 10 days at 25°C, dishes showing good fungal growth were selected for the experimental inoculation.

Isolation from infected cadavers

Sabouraud's Maltose Agar supplemented with 1% Yeast extract (SMAY) medium was used to

isolate the fungus. Conidia of the *N. rileyi* formed on the cadavers were taken by a mycological loop and streaked on SMAY medium. After incubation at room temperature 28 ± 2°C for a week, the colonies obtained were transferred to SMAY slants for preservation. The isolates were identified by microscopic view and observed for the conidia forming mycelia for conidiogenous structure and conidial morphology (Samson *et al.*, 1988; Aoki, 1989). *Nomuraea rileyi* isolates were stored at refrigerated condition (4° C).

CULTURAL CHARACTERISTICS OF *N. rileyi*

Effect of different carbon sources on cultural characteristics

The media prepared from six different carbon sources *viz.*, sucrose, fructose, glucose, maltose, carboxyl methyl cellulose and starch 20 g L^{-1} along with potato extract (200 g L^{-1}) and agar (20 g L^{-1}). Observations on radial growth, biomass and spore count were taken as follows.

a. Radial growth

The growth rate of mycelia in terms of colony diameter (mm) was measured on solid medium. A fungal disc measuring 0.5 cm of the respective isolate before sporulation was inoculated at the centre of the plate. The inoculated agar plates were incubated at 25 ± 1°C for 15 days. Growth of the colony dia meter in mm was measured at an interval of 5 days (Hall and Bell, 1961).

b. Biomass production and spore count

Fifty ml of medium with different nutrient sources was transferred to 250 ml conical flask in 5 replicates and sterilized 10 mm discs of the fungus grown on the respective mycological medium in petri dishes were transferred to the broth and incubated for 15 days. After incubation at 25 ± 1°C the individual broth cultures were filtered through pre–weight Whatman No. 1 filter papers. The mycelial mats collected on the filter papers weight accounted for the dry mycelial weight or biomass (Hall and Bell, 1961).

Fungal mat was macerated with pestle and mortar using 0.02 per cent Tween 20 (Polyethylene sorbitan monolaurate) as an emulsifier to get uniform spore suspension. Spores were further extracted by passing the suspension through a muslin cloth. The spore count was recorded with the help of improved Neubauer's haemocytometer (Jones, 1962).

RESULTS AND DISCUSSION

The radial growth, biomass and spore production of *N. rileyi* isolates varied significantly with various carbon source media tested and the results are shown in table.

1. PDBC isolate

The potato maltose agar medium recorded maximum radial growth of 51.00 mm. The least radial growth was observed in potato carboxyl methyl cellulose agar medium (35.00 mm). In solid medium, the potato maltose agar medium was significantly superior in spore production which was 3.7 x 10^7 spores per ml followed by potato sucrose agar and potato starch agar (2.1 x 10^7 spores per ml and 2.1 x 10^7 spores per ml respectively), the least sporulation was observed in potato carboxyl methyl cellulose agar (1.1 x 10^7 spores per ml). In liquid medium, the biomass production was found to be higher in potato maltose agar (1.51 g). The lowest biomass yield was recorded potato carboxyl methyl cellulose agar (0.87 g). In liquid medium the maximum spore production was observed in potato maltose agar medium (5.324 x 10^7 spores per ml). The least spore production was recorded in potato carboxyl methyl cellulose agar (1.934 x 10^7 spores per ml) (Table 1).

2. DOR isolate

The data (Table 2) revealed that the maximum radial growth was recorded in potato maltose agar medium (50.41 mm). The minimum radial growth was observed in potato carboxyl methyl cellulose agar (38.00 mm). In solid medium, the potato maltose agar medium was significantly superior in spore production which was 4.1 x 10^7 spores per ml. The least sporulation was observed in potato carboxyl methyl cellulose agar (1.1 x 10^7 spores per ml) (Table 2). In case of liquid media, the maximum biomass

Table 1. Influence of carbon sources in solid and liquid medium on growth and sporulation of *N. rileyi* (PDBC isolate)

Carbon source medium	Solid medium				Liquid medium	
	Radial growth (mm)*			Sporulation on 15th day (10^7 / ml)*	Biomass (gm)*	Sporulation on 15th day (10^7 / ml)*
	5th day	10th day	15th day			
Potato sucrose agar	7.50^{cd}	26.50^{ab}	41.00^{c}	2.107^{b} (0.324)	1.20^{c} (1.094)	3.390^{c} (0.530)
Potato fructose agar	7.75^{c}	21.50^{d}	39.50^{d}	1.907^{d} (0.280)	1.00^{e} (0.989)	2.326^{e} (0.366)
Potato glucose agar	7.15^{de}	26.00^{b}	37.50^{e}	2.009^{c} (0.303)	1.17^{d} (1.080)	3.028^{d} (0.481)
Potato maltose agar	10.50^{a}	27.00^{a}	51.00^{a}	3.725^{a} (0.571)	1.51^{a} (1.228)	5.324^{a} (0.726)
Potato carboxyl methyl cellulose agar	7.00^{e}	17.50^{e}	35.00^{f}	1.102^{e} (0.166)	0.87^{f} (0.933)	1.934^{f} (0.286)
Potato starch agar	8.25^{b}	25.00^{c}	43.00^{b}	2.105^{bc} (0.323)	1.43^{b} (1.195)	3.831^{b} (0.583)
CD (P = 0.05)	0.4358	0.8321	0.5742	0.0023	0.0044	0.0093
SEd	0.2000	0.3819	0.2635	0.0010	0.0020	0.0043

* Values are mean of five replications; Figures in parentheses represent log transformation (spore yield) and square root transformation (biomass); Means in a column followed by same superscript letters are not significantly different according to DMRT at P = 0.05; PDBC – Project Directorate of Biological control, Bangalore.

Table 2. Influence of carbon sources in solid and liquid medium on growth and sporulation of *N. rileyi* (DOR isolate)

Carbon source medium	Solid medium				Liquid medium	
	Radial growth (mm)*			Sporulation on 15th day (10^7 / ml)*	Biomass (gm)*	Sporulation on 15th day (10^7 / ml)*
	5th day	10th day	15th day			
Potato sucrose agar	5.16^{c}	15.50^{d}	42.00^{c}	2.068^{c} (0.315)	1.32^{c} (1.148)	3.701^{c} (0.570)
Potato fructose agar	4.66^{c}	12.66^{f}	40.00^{d}	1.768^{d} (0.247)	1.01^{e} (1.004)	2.347^{e} (0.370)
Potato glucose agar	5.00^{b}	16.50^{c}	39.16^{dc}	2.033^{c} (0.308)	1.15^{d} (1.070)	3.645^{d} (0.562)
Potato maltose agar	8.66^{a}	23.83^{a}	50.41^{a}	4.101^{a} (0.613)	1.63^{a} (1.277)	6.819^{a} (0.820)
Potato carboxyl methyl cellulose agar	3.86^{d}	14.00^{e}	38.00^{e}	1.101^{e} (0.042)	0.80^{f} (0.872)	1.900^{f} (0.278)
Potato starch agar	4.81^{c}	21.66^{b}	48.16^{b}	2.908^{b} (0.464)	1.50^{b} (1.224)	4.319^{b} (0.635)
CD (P = 0.05)	0.5296	0.8895	1.5933	0.0127	0.0036	0.0196
SEd	0.2431	0.4082	0.7312	0.0058	0.0017	0.0090

* Values are mean of five replications; Figures in parentheses represent log transformation (spore yield) and square root transformation (biomass); Means in a column followed by same superscript letters are not significantly different according to DMRT at P = 0.05; DOR – Directorate of Oilseeds Research, Hyderabad

was recorded in potato maltose agar (1.63 g). The minimum biomass productions of 0.80 g were observed in potato carboxyl methyl cellulose agar media. In liquid media the maximum spore production was observed in potato maltose agar medium (6.819 x 10^7 spores per ml). The minimum spore production was observed in potato carboxyl methyl cellulose agar (1.900 x 10^7 spores per ml) (Table 2).

3. Coimbatore isolate

The excellent growth of the fungus was obtained with potato maltose agar (42.00 mm). Potato carboxyl methyl cellulose agar recorded comparatively lesser growth of 30.00 mm. In solid medium, potato maltose agar was found significantly superior than other media by recording abundant sporulation of 3.3 x 10^7 spores per ml and potato sucrose agar produced comparatively lower sporulation (1.4 x 10^7 spores per ml). The maximum biomass was recorded in potato maltose agar medium (1.50 g). The minimum biomass production of 0.90 g and 0.70 g were observed in potato fructose agar and potato carboxyl methyl cellulose agar media. In liquid media, the maximum spore production was observed in potato maltose agar medium (5.1 x 10^7 spores per ml). The spore production was significantly reduced and the least spore yield was recorded in potato carboxyl methyl cellulose agar with 1.9 x 10^7 spores per ml (Table 3).

Different carbon sources media tested, potato maltose agar was significantly superior for *N. rileyi* over all other media and supported the maximum biomass, mycelial growth and conidial count. This is in corroboration with the result of Vimala Devi and Prasad (1994) who reported that maltose and sucrose as good carbon sources for sporulation of *N. rileyi*. Earlier various carbon sources tested were corn flour, corn starch, rice hull, glycerol and sucrose (Mazumdar *et al.*, 1995). Vimala Devi (1995)

Table 3. Influence of carbon sources in solid and liquid medium on growth and sporulation of *N. rileyi* (Local isolate)

Carbon source medium	Solid medium				Liquid medium	
	Radial growth (mm)*			Sporulation on 15th day (10^7 / ml)*	Biomass (gm)*	Sporulation on 15th day (10^7 / ml)*
	5th day	10th day	15th day			
Potato sucrose agar	5.00^{ab}	15.83^{b}	38.00^{c}	1.400^{f} (0.146)	1.14^{c} (1.065)	2.940^{c} (0.468)
Potato fructose agar	4.66^{c}	14.50^{c}	33.50^{e}	1.515^{d} (0.180)	0.90^{d} (0.987)	2.380^{d} (0.377)
Potato glucose agar	4.83^{bc}	13.33^{d}	35.50^{d}	2.006^{e} (0.301)	1.14^{c} (1.067)	2.998^{c} (0.477)
Potato maltose agar	5.33^{a}	17.50^{a}	42.00^{a}	3.323^{a} (0.523)	1.50^{a} (1.219)	5.139^{a} (0.711)
Potato carboxyl methylcellulose agar	4.50^{c}	15.00^{c}	30.00^{f}	1.475^{e} (0.168)	0.70^{e} (0.835)	1.906^{e} (0.279)
Potato starch agar	5.51^{a}	17.41^{a}	40.10^{b}	2.713^{b} (0.433)	1.40^{b} (1.176)	3.9950^{b} (0.601)
CD (P = 0.05)	0.4248	0.8120	0.9564	0.0058	0.0091	0.0100
SEd	0.1946	0.3727	0.4390	0.0026	0.0042	0.0046

* Values are mean of five replications; Figures in parentheses represent log transformation (spore yield) and square root transformation (biomass); Means in a column followed by same superscript letters are not significantly different according to DMRT at P = 0.05; Local isolate collected from Coimbatore (Thondamuthur).

reported that various carbon sources such as soluble starch, corn starch or malt extracts were additionally used with a protein base medium to increase the growth and sporulation of *N. rileyi*. To optimize the growth and sporulation, the carbon, nitrogen, mineral and pH levels may require the precise balancing (Gupta and Mukerjii, 2000). Tripathi (2006) reported that all the carbon sources amended on PDA supported the mycelium growth, however, maltose was found to be significantly superior in increasing the growth followed by glucose.

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