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Studies on *in vitro* Growth Rate of Culturable Gut Bacterial Flora of Three Stem Borers Infesting Rice (*Oryza sativa*)

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Abstract

Stem borers are the most devastating pest of rice globally. The present experiment was conducted to compare the *in vitro* growth rate under different temperature conditions of culturable gut bacteria isolated from three different rice stem borers *viz.*, *Scirpophaga incertulas* (YSB), *Chilo suppresalis* (SSB) and *Sesamia inferens* (PSB). Growth was recorded spectrophotometrically by measuring optical density at 660 nm. The gut bacterial consortia of YSB and PSB grew better than those of SSB at lower temperatures (15 and 20 °C) while that of PSB performed equally well in the entire range of temperatures. The gut bacterial consortium of SSB grew luxuriantly with the increase in temperature with the peak growth at 30 °C. There was no marked difference in the growth trend of all the bacterial isolates at 30 °C. These results indicate differential temperature preferendum of gut flora and give insights into their possible relation with behavioural response of the host insect species at different temperature.

Keywords: Growth rate, Gut bacteria, Host insect, Oryza sativa, Rice, Stem borers

Introduction

Since ages, rice has been an integral element of the cultural identities of several communities around the world (Prasad *et al.*, 2017) and around half of the global population relies on rice for meeting their nutritional and caloric requirements (Pathak *et al.*, 2018). It is also an important forex earner and crucial to the economies of many nations (Dhakal and Poudel, 2020). Yield of even the improved varieties of rice is constrained by several biotic factors and arthropod pests are one of the major stresses amongst them. Out of all the rice insect pests, stem borers are regarded as the most dangerous worldwide (Ane and Hussain, 2016). Globally, six species of stem borers, *viz.*, *Scirpophaga incertulas* (Walker) [Yellow stem borer]; *S. innotata* (Walker) [White stem borer]; *Chilo suppressalis* (Walker) [Striped stem borer]; *C. polychrysus* (Meyrick) [Dark headed stem borer]; *C. auricilius* (Dudgeon) [Gold fringed stem borer] and *Sesamia inferens* (Walker) [Pink stem borer], attack rice crop both at vegetative stage causing dead heart and at reproductive stage leading to white ear head (Cantindig and Hoeng, 2003). Stem borer has accounts for around 30% of the annual yield losses in rice at the national level (Dhaliwal and Arora, 2009; Krishnaiah and Varma, 2018).

Insect alike other animals form association with various microbes which may be mutualistic and commensal to pathogenic (Hosokawa and Fukatsu, 2020). Depending upon their functions, they are categorised into primary endosymbionts (Jing *et al.*, 2020) and secondary endosymbionts (Douglas, 2015). Symbionts have been reported to cater important roles in a range of physiological activities in insects including digestion, nutrition, reproduction, defence, behavioural manipulation, enhanced resilience to climate change, heat tolerance, *etc.* (Engel and Moran, 2013; de Almeida *et al.*, 2017; Shukla *et al.*, 2018). Adaptation

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of the host insect is highly influenced by the symbiotic bacteria (Jang and Kikuchi, 2020; Li *et al.*, 2020) and they are particularly prominent in the digestive tract (Engel and Moran, 2013).

Thorough literature review revealed no prominent work elucidating any growth rate studies of gut bacteria of stem borers. So, the present study aimed at isolation of gut bacteria from rice stem borers and compare their growth rate *in vitro* under different temperature conditions. This study may be instrumental in better understanding of the temperature preferendum of gut flora and will give insights into their possible relation with behavioural response of the host insect species at different temperature.

Material and Methods

Culture-Dependent Isolation of Gut Bacteria

Considering the relative abundance and severity of damage in pan India context, the present study on the gut bacteria was confined to the three main stem borers of rice, *viz.*, *Scirpophaga incertulas* (YSB), *Chilo suppresalis* (SSB) and *Sesamia inferens*(PSB). The larvae of the above-mentioned borers were collected from the experimental farm of ICAR-National Rice Research Institute, Cuttack, Odisha. The variety Varshadhan (CRLC 899) was sampled for live *S. incertulas*, *S. inferens* and *C. suppresalis* larvae at the same time from the same field. The larvae feeding inside white ears were collected by dissecting the infested rice tillers in the laboratory and used for further experiments.

The gut bacteria were isolated following the procedure of Orozco-Flores *et al.* (2017) with slight modifications. Live 3rd and 4th instar larva of the collected stem borers were surface sterilised with 0.1% sodium hypochlorite, 70% ethanol and distilled water in succession to eliminate adherent contaminants prior to gut dissection. Three larvae of each species were selected and the head and the last uromere were seperated with an alcohol-sterilised scalpel and the digestive tract was carefully pulled out from the larva with the aid of forceps. Excised gut was homogenized in sterilized Eppendorf tubes containing sterile distilled water using sterile micro-pestle and then serially diluted from 10^{-1} to 10^{-6}. Suspensions from each dilution were spread on Nutrient Agar (NA) plated petri dishes. After a 24-48 hours incubation period at 27±1 °C, visually distinct colonies were identified, picked and streaked on NA plates using sterilized inoculation loop inside laminar airflow cabinet under aseptic conditions. Streaking was done four to five times to obtain pure cultures.

Growth Studies of Isolated Gut Bacteria

The bacterial isolates were inoculated individually into sterile tubes containing 10 ml of nutrient broth. Tube containing only nutrient broth served as control. Three such sets were prepared each replicated thrice and incubated at 15 °C for 24, 48 and 72 hours respectively. Growth test data was recorded spectrophotometrically (UV-Vis Thermo, Model: Evolution 300) by measuring absorbance (optical density) at 660 nm (OD_{660}) at the end of the incubation period. The experiment was repeated with similar conditions at 20, 25 and 30 °C. Similarly, the experiment was performed for the culturable gut bacterial consortia of YSB, PSB and SSB. The growth rate was estimated by plotting the line graphs for time versus OD_{660} values (Ramya *et al.*, 2016).

Table 1: Impact of temperature on the growth of culturable gut bacteria isolated from larvae of stem borer complex

Isolate code	Effect of temperature (°C) on growth* of culturable gut bacteria at different time intervals (hours)											
	15			20			25			30		
	24	48	72	24	48	72	24	48	72	24	48	72
YSB_1	0.256	0.341	0.571	0.234	0.308	0.368	0.187	0.249	0.258	0.205	0.357	0.307
YSB_2	0.143	0.298	0.416	0.166	0.263	0.344	0.194	0.210	0.222	0.153	0.347	0.250
YSB_3	0.112	0.093	0.156	0.053	0.066	0.071	0.046	0.086	0.105	0.070	0.156	0.019
YSB_4	0.368	0.311	0.280	0.213	0.233	0.264	0.114	0.147	0.198	0.166	0.268	0.318
YSB_5	0.022	0.037	0.037	0.015	0.018	0.013	0.004	0.067	0.041	0.028	0.282	0.212
PSB_1	0.235	0.225	0.305	0.141	0.208	0.267	0.151	0.209	0.240	0.160	0.344	0.221
PSB_2	0.031	0.038	0.046	0.012	0.019	0.028	0.008	0.044	0.037	0.053	0.218	0.213
PSB_3	0.233	0.355	0.320	0.220	0.282	0.359	0.188	0.262	0.287	0.193	0.353	0.303
PSB_4	0.310	0.176	0.193	0.194	0.184	0.138	0.128	0.213	0.129	0.170	0.214	0.251
PSB_5	0.334	0.409	0.411	0.272	0.369	0.355	0.208	0.235	0.327	0.257	0.417	0.308
PSB_6	0.184	0.288	0.295	0.080	0.144	0.160	0.108	0.119	0.183	0.130	0.277	0.156
SSB_1	0.153	0.239	0.116	0.099	0.122	0.231	0.095	0.141	0.082	0.170	0.293	0.411
SSB_2	0.150	0.170	0.194	0.223	0.189	0.238	0.074	0.223	0.253	0.146	0.364	0.360
SSB_3	0.514	0.109	0.177	0.047	0.055	0.074	0.041	0.073	0.092	0.096	0.140	0.013
SSB_4	0.079	0.120	0.097	0.141	0.244	0.212	0.031	0.070	0.083	0.156	0.202	0.052
SSB_5	0.170	0.139	0.094	0.179	0.115	0.137	0.242	0.232	0.190	0.138	0.220	0.306

[*OD values at 660 nm]

Results and Discussion

A total of 16 visually distinct bacterial biota were isolated from the gut of the three rice stem borers by culture dependent approach consisting of five distinct isolates from yellow stem borers (YSB), six distinct isolates from pink stem borers (PSB) and five distinct isolates from striped stem borer (SSB).

The mean of the three replications of OD (optical density) values recorded after different incubation period, *i.e.*, 24, 48 and 72 hours for each sample has been tabulated in table 1 for all the different temperatures, *i.e.*, 15, 20, 25 and 30 °C.

A comprehensive analysis of the table 2 and figure 1 reveals a clear picture about the growth of the bacterial consortia of the three stem borer species. Comparison of the mean OD values of the mixed cultures of bacterial isolates from YSB, PSB and SSB at different temperatures indicated that at lower temperatures; say, 15 and 20 °C; the gut bacterial consortia of YSB and PSB perform better than those of SSB. The bacterial consortium of PSB had grown equally well in the entire range of temperatures. However, the bacterial microbiota from SSB which failed to grow exponentially at 15 °C, showed luxuriant growth with the increment in temperatures with the highest performance being recorded at 30 °C. At 30 °C the bacterial microbiota from all the stem borers showed more or less a similar growth trend.

Amongst the YSB isolates, YSB 1 showed the most efficient growth in all temperatures with best growth at 15 °C. At 15 °C, YSB 2 also showed overall good growth and YSB 4 recorded highest growth at 30 °C. PSB 5 showed consistently good growth in all the temperatures. Out of the SSB isolates, though SSB 1 showed highest growth at 30 °C but SSB 2 performed well in other temperatures too.

The relevance of microbial symbiosis has been pointed out by several researchers in various insects (Feldhaar, 2011; Wong *et al.*, 2015; Jing *et al.*, 2020). The gut bacterial consortia of YSB and PSB perform better than those of SSB at lower temperatures like 15 and 20 °C. So, this differential

Table 2: Impact of temperature on the growth of culturable gut bacterial consortia of different stem borers

Gut bacterial consortia	Effect of temperature (°C) on growth* of culturable gut bacterial consortia at different time intervals (hours)											
	15			20			25			30		
	24	48	72	24	48	72	24	48	72	24	48	72
YSB	0.170	0.223	0.302	0.142	0.183	0.229	0.106	0.152	0.162	0.148	0.284	0.224
PSB	0.209	0.236	0.267	0.151	0.199	0.237	0.131	0.177	0.193	0.162	0.298	0.247
SSB	0.198	0.162	0.141	0.142	0.153	0.182	0.110	0.146	0.156	0.143	0.251	0.221

[*OD values at 660 nm]

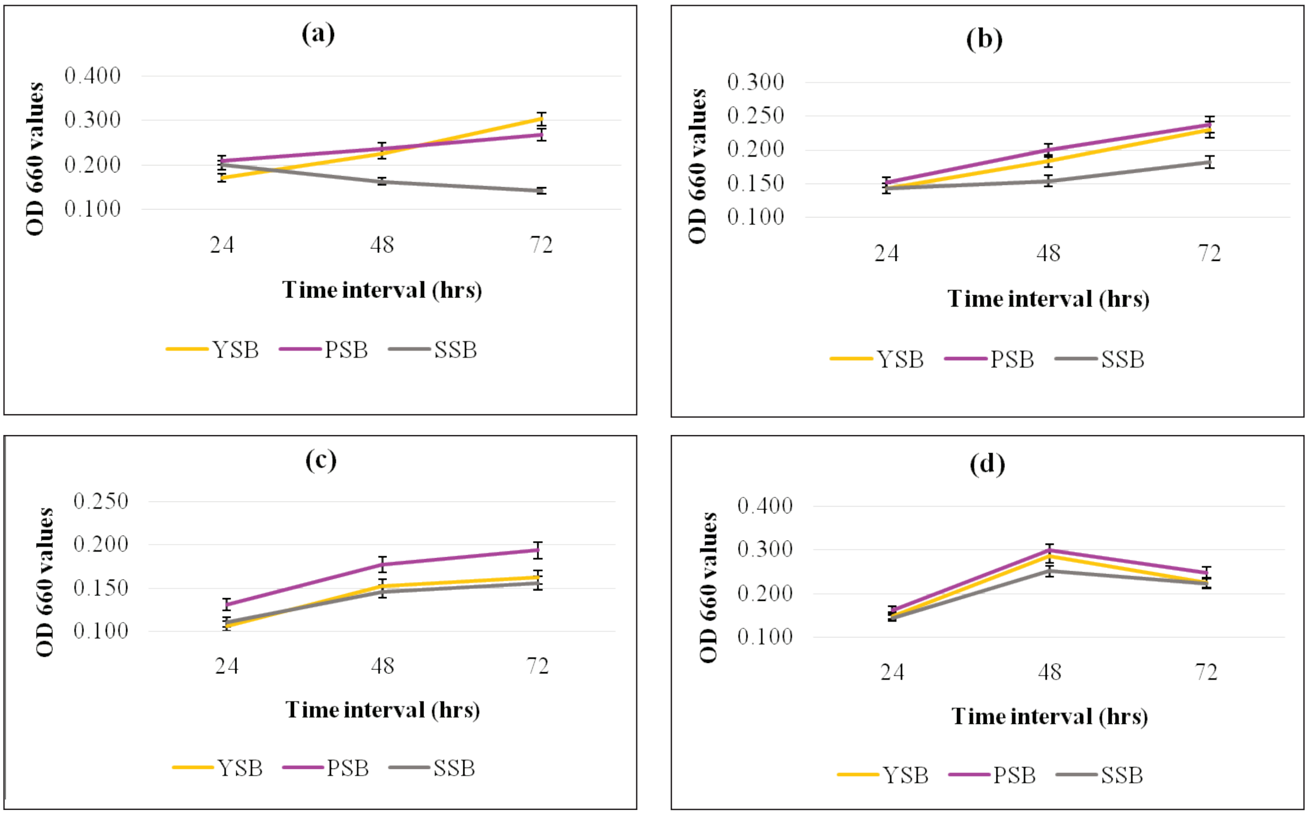

Figure 1: Growth curve of the gut bacterial consortia of YSB, PSB and SSB at: (a) 15 °C, (b) 20 °C, (c) 25 °C and (d) 30 °C

temperature preferendum of the gut bacteria may be correlated with the emergence pattern of the stem borers (McLean *et al.*, 2016; Itoh *et al.*, 2018). In the Boro/ summer (*Rabi*) rice, the YSB infestation occurs earlier when the ambient temperature is quite low followed by PSB. However, as the temperature increases the abundance of YSB subsides and the SSB starts to emerge. So, a possible influence of the gut bacterial microbiota on stem borers' emergence pattern may be operational as effect of gut symbionts on host insect behaviour and response to climate have been reported by previous researchers (de Almeida *et al.*, 2017; Liberti and Engel, 2020). However, it is often difficult to confirm whether the host behavioural response is attributable to symbionts' manipulation or due to host's adaptive response (Hosokawa and Fukatsu, 2020) and therefore, this study opens up avenues for further research for interpreting this interesting biological phenomenon.

Conclusion

The different species of stem borers although infesting the same variety of rice in the same cropping season; harboured visually distinct bacterial isolates in their gut. All these bacterial isolates performed differently with regards to *in vitro* growth rate in NA medium. The gut bacteria of YSB and PSB showed comparatively better growth than those of SSB at lower temperature. However, at higher temperature there was no significant difference in the growth trend of all the bacterial isolates. This study confirms that there is marked difference in the growth rate of gut bacteria isolated from three different species of stem borers although infesting the same rice variety at the same time and collected from the same habitat.

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