soft and fragile, and liquefaction of body tissues inside the body cavity further adds to the tenderness of the infected insect. The chitinous cuticle of the insect which virtually covers all external surfaces, even extending through the foregut, hindgut and tracheal tubes constituting the first line of passive defence in insects[18].

The overall destruction of tissues led to liquefied contents inside the body cavity, giving the infected insect a turgid appearance. The infected larval body is laden with polyhedral occlusion bodies (POBs) which contain virions. Even a slight damage or disturbance of the integument released liquefied body fluid containing large number of POBs. This infected fluid further spread infection when healthy larvae came in contact with the fluid, causing autoinfection[15].

In the histomicrograph of infected insect, deposition of millions of Polyhedra was observed on the body wall, in the crypts of the body wall and inside the tissues (Figure 2 *c–e*). After being flooded with NPV, the protein content of the haemolymph is reduced and thus structural properties of the cuticle are affected leading to fragile skin and liquefied body fluid. The larvae become turgid and sluggish that they are unable to move.

To conclude, the histopathological studies have revealed that midgut epithelium is the principal target tissue for action of NPV. However, it was also observed in the histomicrographs that extensive tissue destruction occurred in the body and epithelium of the insect. These damages were from the centre towards the periphery, which was also evident by the development of various morphological and behavioural abnormalities developed after the insect was severely infected.

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Antiviral property of marine actinomycetes against White Spot Syndrome Virus in penaeid shrimps

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Aquaculture farms, particularly in Southeast Asia are facing severe crisis due to increasing incidences of White Spot Syndrome Virus (WSSV). Actinomycetes have provided many important bioactive compounds

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of high prophylactic and therapeutic value and are continually being screened for new compounds. In this communication, the results of a study made to determine the effectiveness of marine actinomycetes against the white spot disease in penaeid shrimps are presented. Twenty-five isolates of actinomycetes were tested for their ability to reduce infection due to WSSV among cultured shrimps. When these actinomycetes were made available as feed additives to the post-larvae of the black tiger shrimp *Penaeus monodon* for two weeks and challenged with WSSV, the post challenge survival showed variations from 11 to 83%. However, six isolates have shown to be the most potential candidates for further study.

Keywords: Actinomycetes, aquaculture farms, penaeid shrimps, White Spot Syndrome Virus.

AQUACULTURE can provide an effective solution to the world's increasing demand for proteinacious food, but the sustained production is being hampered primarily by the outbreak of diseases. The shrimp culture industry is currently going through a period of severe crisis due to the outbreak and recurrence of a viral infection, commonly referred to as the White Spot Syndrome Virus (WSSV)[1]. In cultured shrimps, WSSV infection can cause a cumulative mortality of up to 100% within 3–10 days, thereby causing considerable economic loss to the shrimp farmers. The virus infection first appeared among the culture ponds and was discovered in Taiwan, from where it quickly spread to other shrimp-farming countries in Southeast Asia[2]. In India, WSSV infection was first reported from the Kandaleeru creek-fed shrimp farms in Andhra Pradesh[3] and was subsequently reported from ponds located all along the Indian coasts[4]. WSSV has a double-stranded DNA and is an enveloped, ovoid-shaped virus with a rod-shaped nucleocapsid having flat ends[5,6]. A well-formulated strategy is needed to control this disease.

Use of aquatic plants and animals for biomedical research and the potential of lower marine organisms as sources of pharmaceuticals have opened up new vistas to the whole scenario of aquaculture activities. Recently, Achuthankutty and Desai[7] have described a patented leaf extract formulation that is effective as a prophylactic and therapeutic agent against WSSV in penaeid shrimps. Although actinomycetes constitute only <0.05% of the culturable microbial community[8], they have provided many important bioactive compounds[9] and many of them have exhibited antiviral activity[10,11]. However, no studies on the anti-WSSV properties of marine actinomycetes have been made so far. Therefore, a study was undertaken for screening marine actinomycetes for anti-WSSV property and the results are presented in this communication.

Twenty-five isolates of actinomycetes were randomly selected (source: Microbiology Laboratory, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin, India) for the study. These isolates were isolated from the coastal waters of Cochin (southwest coast of India) and purified by repeated streaking on marine actinomycete growth (MAG) medium (starch – 1 g, yeast extract – 0.4 g, peptone – 0.2 g, agar – 2 g, sea water – 50 ml, pH – 7) plates and the colonies were individually isolated by plating. Actinomycetes were first inoculated onto seed medium (5 ml of MAG) and then transferred to production medium (glycerol – 2.5 g, beef extract – 0.5 g, peptone – 0.5 g, yeast extract – 1.0 g, $MgSO_4.7H_2O$ – 0.05 g, K_2HPO_4 – 0.05 g, $CaCO_3$ – 0.1 g, sea water – 50 ml, pH – 7) to increase the cell density for increased production of bioactive compounds. After 10 days of incubation, the fermentation broth was concentrated in a vacuum evaporator (Speed Vac, Savant, USA) and 100 ml of the broth was further concentrated to 5 ml and incorporated into 10 g of the pellet feed (Higashimaru, Cochin) with the help of a binder (Bindex gel). Thus, twenty-five feeds were prepared with twenty-five selected isolates of actinomycetes. Two types of control feeds were used for the study. They were designated as C_1 (pellet feed incorporated with production media) and C_2 (pellet feed without any additives).

Penaeus monodon post larvae (PL-20) ranging in weight from 0.015 to 0.03 g were used for the study. PL were obtained from Matsyafed Hatchery (Ponnani, Kerala). They were PCR-screened and found to be negative for WSSV. The PL were acclimatized for one week under laboratory conditions by feeding pellet feed (Higashimaru). Twenty-five acclimatized PL were introduced into each experimental tank and were fed on their respective experimental diet and reared for two weeks. Three replicates were maintained for each feed. Mortality during this period was observed to be negligible in all the tanks.

Fibre reinforced tanks (30 l capacity) were used for the experiment. Water quality was monitored daily[12] and maintained as shown in Table 1. On alternate days, 50% water was exchanged from the experimental tanks after removing the faeces and unconsumed feed.

The life span of haemocyte in shrimps is about two weeks[13]. Therefore, after feeding on experimental diet for

Table 1. Rearing conditions and water quality

Initial body weight (average)	0.02166 g
Stocking density	25 PL/tank
Tank capacity	30 l
Volume of water	15 l
Feeding level	15–20% body weight
Feeding frequency	Twice daily
Experimental duration	22 days
Water temperature	24–27°C
pH	7.5–8
Salinity	24–26 ppt
NH_3	0.01–0.02 mg/l
NO_3	Below detectable level
NO_2	0.001–0.01 mg/l
Dissolved oxygen	6–7 mg/l

two weeks, they were challenged with WSSV. This was done by feeding the PL with WSSV-infected adult *P. monodon* (confirmed by PCR screening), after starving them for 12 h. They were fed in the morning and evening *ad libitum*, ensuring availability of infected meat to the entire experimental PL in the tanks. After the challenge, they were fed on the respective experimental diet. Triplicates were maintained for each diet and rearing conditions were maintained as given in Table 1. Mortality was recorded everyday and the mortality caused by WSSV infection was confirmed by checking the characteristic cuticular white spots on the carapace. The infected PL exhibited symptoms characteristic of WSSV. They appeared sluggish, surfaced frequently, showed lack of appetite and developed reddish discoloration and white spots on the inner margin of the carapace and the moribund individuals exhibited reduced preening activity[14].

The data were subjected to Duncan's multiple range analysis using the SPSS 10.0 package for Windows to bring out the differences between various treatment means.

The mortality rate in the controls sharply increased from day 3 and among PL fed on SL 80, SL 97, SL 105 and SB 301 diet. As the disease intensified, almost complete mortality occurred in the controls. Maximum death occurred on day 4 in tanks fed on SL 39, SL 37, SB 377 and on day 5 in those fed on SL 100, SL 85, SL 35, SL 25, SL 20, SL 4, SB 361, SA 20, SA 17, SA 14B, SA 14A and SA 9 diet. In the case of SL 33, SL 6, SA 99, SA 8 and SA 2 fed PL, increased mortality occurred on day 6. However, in tanks fed with SL 27, the population remained nearly stable throughout the period of the experiment.

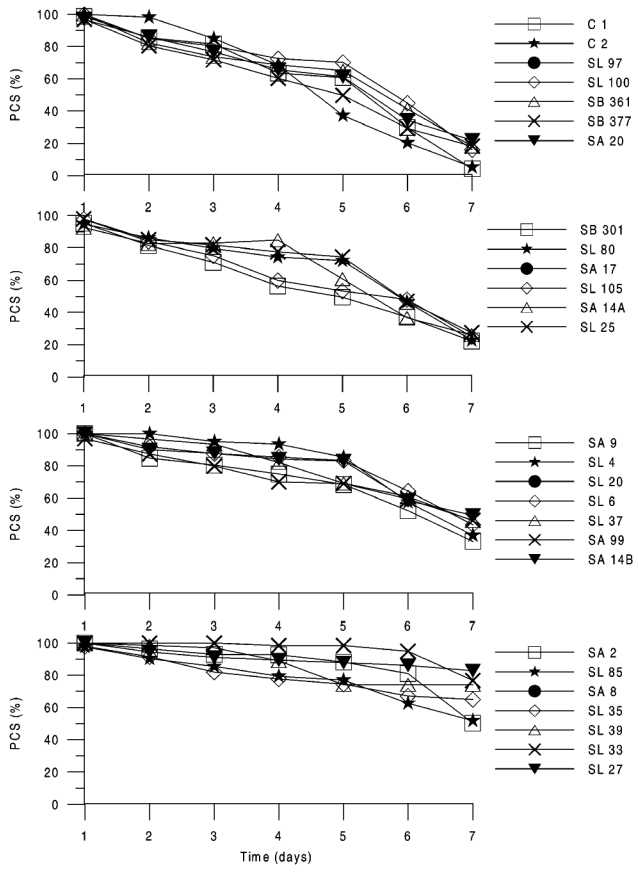

Figure 1. Post challenge (White Spot Virus) survival of *Penaeus monodon* post larvae fed on various experimental feeds.

The pattern of post-challenge survival % (PCS%) in the twenty-seven treatments (twenty-five experimental and two controls) exhibited a wide range of variation during the course of the experiment (Figure 1). PCS% was lowest in the controls C_1 – 4.3 and C_2 – 5.2 on day 7. PL fed on SL 100, SL 97, SA 9, SA 14 A, SA 17, SA 20, SB 301, SB 361, SB 377, SL 25, SL 89, SL 80 and SL 105, exhibited PCS% that varied between 10 and 25. A higher PCS% varying from 30 to 40 was observed among PL fed on SA 14B, SA 99, SL 4, SL 20, and SL 37. However, six feeds, viz. SA 2, SA 8, SL 27, SL 33, SL 39 and SL 85 recorded the highest PCS% ranging between 50 and 83. Also, severity of the infection observed on days 3, 4 and 5 in PL fed with other diet was not visible in these groups. Therefore, these six isolates can be considered as the most potential ones against WSSV infection.

When the data were subjected to Duncan's multiple range analysis (Table 2) to bring out the differences between treatment means, thirteen groups emerged. C_1, C_2,

Table 2. Post challenge (White Spot Virus) survival of *Penaeus monodon* post larvae fed with different experimental feeds and controls. (Isolates having same superscripts formed one group)

Isolate	Day 7	SD
$SA2^{hij}$	50.18	4.65
$SA8^{ijk}$	60.00	7.10
$SA9^{efg}$	33.18	12.62
$SA14A^{cdef}$	26.01	7.26
$SA14B^{hij}$	49.46	0.93
$SA17^{cdef}$	24.44	5.09
$SA20^{cde}$	22.08	6.07
$SA99^{ghij}$	46.37	6.44
$SB301^{cde}$	22.22	10.18
$SB361^{bcd}$	18.21	2.47
$SB377^{abc}$	18.30	8.49
$SL4^{fgh}$	36.98	6.50
$SL6^{ghi}$	43.60	4.39
$SL20^{ghi}$	41.90	7.66
$SL25^{def}$	27.49	5.21
$SL27^{m}$	82.78	9.48
$SL33^{lm}$	76.70	7.27
$SL35^{kl}$	64.95	8.06
$SL37^{ghi}$	46.03	8.50
$SL39^{lm}$	74.13	8.78
$SL80^{cde}$	22.77	9.34
$SL85^{iij}$	51.76	7.80
$SL97^{abc}$	11.67	10.41
$SL100^{abc}$	16.06	1.57
$SL105^{cdef}$	25.07	7.22
Cntrl 1^{abc}	4.30	7.45
Cntrl 2^{abc}	5.26	9.12

SL 97 and SL 100 formed one group, as they did not show any significant variation. All the other isolates formed different groups depending on the similarities. In the study, although six isolates recorded PCS greater than 50%, Duncan's multiple range analysis brought out further variations and grouped them into three different groups, viz. SA 2, SA 8, SL 85 as one, SL 39 and SL 33 as the second and SL 27 as the third group.

Flegel[15] reviewed the response of shrimps to viral pathogens and suggested that their defence system can be stimulated for a limited length of time, which is often much shorter than that in vertebrates. Similar results have also been obtained in a study by Alabi *et al.*[16]. Results of several defence stimulation experiments followed by WSSV challenge tests are available. For instance, antigens that can be absorbed by shrimp via the digestive system can contribute to disease resistance in shrimp[17]. Dietary-glucans also have been shown to retard WSSV infection in *P. monodon*[18]. Other prophylactic components that could delay WSSV infection in *P. japonicus* were peptidoglycan and lipopolysaccharide (LPS), both bacterial cell-wall components, and fucoidan, an algal polysaccharide[19,20]. In another study, oral administration of LPS at the rate of 20 μg LPS per kg shrimp body weight^{-1} day^{-1} for 7 days against penaeid acute viraemia (PAV) resulted in 75% survival[20]. In the present study, six isolates have resulted in >50% survival after feeding for 7 days and three of them >70%. Therefore, the potential of marine actinomycetes in increasing the survival of WSSV-infected shrimp appears to be more.

Pentalactones isolated from the fermentation broth of *Streptomyces* sp. M-2718 has been reported to be active against several DNA viruses[11]. The antiviral activities of pentalactones and pyrrole-2-carboxylic acid against herpes simplex virus had already been assayed and described[21]. Researchers have reported that guanine-7-N-oxide produced by *Streptococcus* sp. was found to inhibit *in vitro* replication of the fish herpes virus (Onchorhynchus Masou Virus), rhabdovirus (Infectious Hematopoitic Virus) and a bisegmented double-stranded fish virus (Infectious Pancreatic Necrosis Virus)[10]. All these studies indicate that, the bioactive compounds produced by actinomycetes function as antiviral antibiotics. The results obtained in the present study may also be considered in accordance with these findings. Thus, it leads to the obvious conclusion that isolates of actinomycetes in the culture broth may have produced bioactive compounds that possess potent antiviral activities. These isolates, when incorporated in the feeds, lowered WSSV infection in shrimps. Isolation of these compounds and their characterization are essential to further the findings of this study, which would lead to the possibility of developing antiviral agents effective against white spot disease in shrimps.

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Discrete generation cycles in the tropical moth *Opisina arenosella*

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Insect populations with discrete generation cycles (DGCs), have been rarely encountered in the tropics. Among the few known species, spatially segregated coastal populations of *Opisina arenosella*, the coconut caterpillar, have been shown to follow DGCs during outbreaks in Sri Lanka. Climatic parameters are known to be important in regulating generation cycles in insect populations. But, unlike temperate conditions, the tropics are characterized by high spatial heterogeneity in climate, which prompted the present investigation on generation cycles of populations of *O. arenosella* occurring in interior dry landscapes of the Indian peninsula. Two spatially isolated populations were regularly sampled for two years and data were subjected to time series analysis to determine periodicity, if any, in the occurrence of different developmental stages of the population. Results showed that populations followed DGC with a periodicity of approximately one generation, and further, correlations showed that there was a definite lead/lag in the peaks of different developmental stages, which closely correspond to the developmental period of different stages of the insect. The findings suggest that discrete cycles of *O. arenosella* may not be related to seasonality. The importance of generation cycles with respect to pest management has also been discussed.

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Keywords: Coconut black-headed caterpillar, generation cycles, host-parasitoid dynamics, insect seasonality.

INSECT populations are known to either cycle with a period of approximately one generation (discrete or non-overlapping generation cycles, DGCs) or all age-classes of the population can occur simultaneously (continuous or overlapping generation cycles). DGCs are common in temperate environments characterized by extensive winters, where populations hibernate through the winter months in a particular developmental stage – as egg, larva or pupa. In other words, winter conditions 'select' a particular developmental stage, which creates uniformity in the surviving population with respect to age, causing the subsequent spring populations or summer populations to follow 'discrete' cycles. In the tropics, however, it is generally believed that lack of such 'selection' can lead to continuous generation cycles in insect populations. Interestingly, certain multivoltine tropical insect species that are active throughout the year are also known to follow DGC. Godfray and Hassell[1] have listed several such species. In India, only *Andraca bipunctata* (Lepidoptera, Bombycidae) has been speculated to be following DGC[1,2].

Populations of *Opisina arenosella* Walker (Lepidoptera, Oecophoridae), commonly called the 'coconut black-headed caterpillar', have been shown to follow partially DGC during outbreaks in Sri Lanka[3]. This species is the major leaf-feeding pest of coconut palms in the entire Indian subcontinent. Surprisingly, earlier studies from India do not refer to generation cycles[4]. *O. arenosella* breeds all year round on coconut palms without undergoing diapause. Although the species infests coconut groves almost throughout peninsular India, it has never been found to occur as a large contiguous population in coconut-growing areas. Infested areas are always interspersed with un-infested ones, suggesting the existence of spatially segregated populations. In India, such populations are distributed over different agro-climatic zones – from high-rainfall coasts[5] to interior dry landscapes[6], which considerably vary from the coastal climate of western Sri Lanka from where an earlier study concluded that the species followed DGCs. Unlike temperate situations, the high spatial heterogeneity of climatic parameters in the tropics would expose different populations of *O. arenosella* to different climatic conditions. These could, directly[7] or indirectly (through their influence on natural enemies)[8], have a differential influence on generation cycles of different populations. Such variation is well illustrated by gypsy moth (*Lymantria dispar*) populations, where the oscillations in periodicity of populations separated by distances of over 1000 km have been shown to be asynchronous[9]. A study was therefore carried out to determine generation cycles with particular reference