Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22, No. (3) : 2020 : 519-527

ISSN-0972-3005

DISRUPTION OF THE *POLYKETIDE SYNTHASE 14* FROM THE FOREST SOIL DWELLING *DICTYOSTELIUM DISCOIDEUM* RESULTS IN A DEFECT IN SLUG FORMATION AND MIGRATION

LAVANYA MUTHUKUMAR [1], MAULD H. LAMARQUE[2] AND KIRAGANDUR MANJUNATH[1]

[1]Department of Microbiology and Biotechnology, Bangalore University, Jnana Bharathi Campus, Bengaluru, Karnataka, 560 056, [2]Université de Montpellier 2, Montpellier, 34095, France.

(Received 1 April, 2020; accepted 2 June, 2020)

Key words : Dictyostelium discoideum*, Soil, Polyketide synthase, Slug size, Slug migration, Dispersion*

Abstract–*Dictyostelium discoideum* is an ecologically important forest soil dwelling social amoeba. It consumes bacteria in decaying matter and maintains the bio-ecological balance in forest soil. When their main nutrient source, bacteria, is scarce, *Dictyostelium discoideum* cells undergo starvation induced development to form dormant spores. *Dictyostelium discoideum* is one of the largest repositories of an important class of enzymes called polyketide synthases (PKS) that make the majority of commercially available antibiotics and therapeutics. However, the in vivo role of these PKS enzymes in the *Dictyostelium discoideum* life cycle and the forest ecology is unknown. In this study the *Dictyostelium discoideum* type I polyketide synthase 14 or *dipks14* gene was disrupted using gene knockout technology. The effect of the loss of the *dipks14* gene on the morphology and behaviour of *Dictyostelium discoideum* was observed. Functional characterization of *dipks14* by gene disruption and phenotyping indicates that the disruption of the *dipks14* correlates with the presence of a mix of non-motile normal sized slugs and motile reduced sized slugs that have a migration defect. *dipks14* gene disruption slugs are able to form fruiting bodies and viable spores, however the distance between the fruiting bodies is reduced. This suggests a role for *dipks14* in maintaining distance between the individual multicellular stages such as the slug and the fruiting body and consequently in uniform spore dispersal and survival of germinating new amoebae ensuring homeostasis of forest soil ecology.

INTRODUCTION

Dictyostelium discoideum is a unicellular eukaryote that belongs to a group of organisms called cellular slime molds or social amoebae. *Dictyostelium discoideum* has a remarkable bimodal life cycle that starts with free living unicellular amoebae that feed on the bacteria in decaying organic matter in the soil (Raper, 1935). Lack of bacterial nutrient source initiates the developmental cycle that eventually results in a multicellular fruiting body (Bonner, 1952). When the bacterial load is low, the amoebae sense a drop in nutrient level and aggregate in groups of 100,000 cells in the wild and form an intermediate migratory slug stage that is approximately 1-2mm in size (Bonner, 2009). The *Dictyostelium discoideum* slug is a collection of cells bound by a cellulosic sheath (Fuchs, Jones and Williams, 1993). Its worm like form is organized into an anterior prestalk and a posterior prespore region (MacWilliams and Bonner, 1979). Slug migration is controlled by the prestalk cells that rotate rapidly within the sheath to propel the slug forwards (Siegert and Weijer, 1992). Motile slugs move away from each other radially from the center when there is no light source, maintaining distance between each slug (Wallraff and Walraff, 1997). The anterior prestalk region also senses light and controls phototactic turning (Fisher Smith and Williams, 1981). In the presence of a light source the slugs migrate directionally towards the light continuing to maintain distance between each other. Once the slugs stop migration they transform into a fruiting body with a stalk and a terminal anterior spore filled spore sac. The prestalk cells differentiate as the stalk of the fruiting body and the prespore cells

differentiate as dormant spores that germinate under favourable conditions to continue the life cycle (Chisholm and Firtel 2004).

Dictyostelium discoideum has 45 putative polyketide synthases (Ghosh *et al.,* 2008; Zucko *et al.,* 2007) and produces several natural product metabolites such as Discadenine, Dictyopyrones and the Polyketides, DIFs and MPBD, differentiation inducing factors and 4-methyl-5-pentylbenzene1,3-diol respectively (Araki and Saito 2019; Manocha 2011). These metabolites are all developmental morphogens and mediate cell to cell signaling leading to cell differentiation and development in *Dictyostelium* . In the developing cell aggregates DIF1 mediates stalk cell fate (Kay *et al.,* 1983) and MPBD mediates spore cell differentiation (Narita *et al.,* 2014). DIF1 and MPBD are products of PKS enzymes DiPKS37 and DiPKS1 respectively. In *Dictyostelium discoideum,* of the 45 putative full length Polyketide Synthases, 39 are predicted to be Type I PKSs (Ghosh *et al.,* 2008). The in vivo roles and product metabolites of these 39 Type I PKS are yet to be characterized.

Naturally occurring Polyketide Synthase enzymes (PKSs) are the major contributors of pharmaceutically important metabolites called Polyketides (Staunton and Weissman, 2001). PKSs are large multi domain proteins which are a part of the secondary lipid metabolism. The multiple domains are used either iteratively or non-iteratively by the PKS enzyme to synthesize a diverse group of lipid metabolites called polyketides (Gokhale, Sankaranarayanan and Mohanty, 2007). Based on whether the domains exist on a single polypeptide or multiple polypeptides PKS enzymes are classified as Type I when domains exist on a single polypeptide, Type II when domains exist on multiple polypeptides, Type III where the same active site performs multiple reactions. Highly reducing type I PKS enzymes have multiple reductive catalytic domains that add hydrogen molecules such as the ER or enoyl reductase and R or reductase domain (Herbst, Townsend and Maier 2018). The three core domains of polyketide synthases are the KS or ketosynthase, AT or acyl transferase and the KR or ketoreductase domains along with the ACP domain (Herbst *et al.,* 2018). Often there are MT or methyltransferase as well as DH or dehydratase domains. The core domains are essential for formation of the polymeric lipid polyketide chain without which the enzyme cannot function. The other domains are optional and may or may not be present.

Many polyketides have therapeutic value for example Erythromycin-A an anti-microbial, Nystatin an anti-fungal, and Avermectin an anti-parasitic (Staunton and Weissman, 2001). In recent years however the focus in Polyketide research has evolved to include investigations into the invivo roles of Polyketide Synthases (Kim *et al.,* 2010; Vats *et al.,* 2012; Funa *et al.,* 2006) as they are over represented in pathogenic Ascomycete Fungi like *Aspergillus* and Actinomycete bacteria like *Mycobacteria*. *Dictyostelium discoideum* is often used as a simple model organism for investigations on multi cellular development (Williams 2010), possess multiple genes homologous to polyketide synthases and has recently been established as a model organism to study the genetics and the biochemistry of polyketide synthases (Austin *et al.,* 2006; Ghosh *et al.,* 2008; Nair *et al.,* 2011). *Dictyostelium discoideum* is therefore a very attractive model to study the in vivo role of PKS genes.

The *Dictyostelium discoideum* Type I PKS genes are highly similar to each other (Muthukumar and Manjunath, 2020; Ghosh *et al.,* 2008). This raises the question of whether they play unique or overlapping roles in the developmental life cycle or ecology of *Dictyostelium discoideum*. DiPKS14 is predicted to be a highly reducing iterative polyketide synthase similar to type I PKSs in pathogenic Fungi and *Mycobacteria* (Muthukumar and Manjunath, 2020). Type I PKS genes have 45% to 95% similarity with the other *Dictyostelium discoideum* PKS genes. To determine the role of DiPKS14 in the life cycle of *Dictyostelium discoideum* a phenotypic and behavioural analysis of the slug stage of the *dipks14* gene knockout strain was undertaken. Directed and undirected slug migration assays were used. We found that the *Dictyostelium disocideum* DiPKS14 *in vivo* function correlated with the migratory slug stage of development. Disruption of DiPKS14 correlated with smaller migrating slugs that could not travel as far the wild-type slugs in the same given time.

MATERIALS AND METHODS

Dictyostelium discoideum growth and development conditions

Dictyostelium discoideum wild-type AX2 cells and *dipks14* gene knockout strains cells were grown in HL5 axenic medium at 22 °C with or without

Blasticidin and harvested by centrifugation at 3000rpm for 3 minutes followed by washes with KK2 buffer. Cells were resuspended at $2x10^8$ cells per ml and plated on non-nutrient agar, cold synchronized at 4 °C and allowed to develop by transferring to 22 °C. Observations of *Dictyostelium discoideum* developmental stages were made using an Olympus microscope.

Genomic DNA extraction

A modified protocol for quick genomic DNA extraction was followed. Media was aspirated from a 90mm plate of *Dictyostelium discoideum* and the cells were washed two times with 1X KK2 buffer. The cells were collected into a sterile 1.5 mL eppendorf using a 1 mL micropipette and spun down at 3000 rpm for 3 minutes in a micro centrifuge. The pellet was resuspended in 500uls of Lysis buffer along with proteinase K and incubated overnight at 55^0C at humid temperatures. 1/10th the volume of sodium acetate at pH 5.2 was added to the above and mixed well. Two to three volumes of ice cold 100% ethanol was added and the contents were mixed by gently turning the tube up and down. The precipitated DNA was spun at 10,000 rpm for 10 minutes and the pellet obtained was washed two times with 70% EtOH. The pellet was air dried for 10 minutes and resuspended in 50uls of TE buffer or water. The resuspended genomic DNA was treated with RNase A (20mg/mL stock at 0.25uls in a 50ul reaction) for 1 hour at 37 °C.

Gel shift PCR to detect the insertion of BSR selection cassette in the *dipks14*ko genomic locus

The following primers were used in this study. rsg1783-5'CGCGCGGCCGCAATGATTTCAAG ATAGGCGATC 3', rsg1784-5'CGCTCTAGACACTA ATCGAATTACTAACATCAAC3', rsg1785-5'CGC AAGCTTCAATTGCACAGCTATTAGATGG3', rsg1786-5'CGCGGTACCCTATTAACATTTACT TGTTTACATTCG3', rsg1895-5'GGACTTTTACCA CCTGAAATGG3',rsg1896-5'CCTAATTTAAA ACTTGA TTGAAGCATC3'. Genomic DNA was extracted from the wild-type and the *dipks14*ko strain. Polymerase chain reaction (PCR) technique was used to amplify fragments of the *Dipks14* genomic locus. PCR was conducted according to manufacturer's instructions for the use of Phusion enzyme. For test PCRs the reaction volumes were typically 25 microlitres and PCR conditions were according to manufacturer's instructions for Taq polymerase from NEB. The template DNA at 30ng concentration was mixed with an excess of 100mM dNTP mix. Forward and reverse primers were added to the reaction mix at 0.4 mM. 1 microlitres of Taq polymerase was added to the PCR reaction mix. Thermal cycling conditions for NEB Taq polymerase were different. Initial denaturation was at 94 °C for 2 minutes, extension was for 1 minute/kb at 72 °C. A Biorad thermal-cycler was used for the PCR reactions.

Slime trail formation assay

Amoebae grown in HL5 media were washed three times in KK2 buffer and resuspended at 2×10^8cells per ml. 10 microlitres of cells were spotted at on water-1% agar plates and allowed to develop for 24hrs in the dark. Slime trails formed were then transferred onto nitrocellulose membranes and stained with coomasie blue.

Quantitation of the zone of migration

Slime trails of wild type and *dipks14* gene disruption slugs in the absence of light were obtained on different parts of the same agar plate, transferred onto nitrocellulose membranes and stained with coomasie blue. The membranes were scanned at the same magnification on an HP scanner. The slugs migrate out radially from the center in the absence of light and therefore the zones were approximately circular in area, therefore the zones of migration were quantified in terms of diameter. The diameter of each zone of migration was quantified using ImageJ and measured in pixels. Each zone was measured four times one each in the vertical, horizontal, left diagonal and right diagonal directions. This confirmed that the zones of migration were approximately circular. The ratio of the diameter of the zones of migration of the *dipks14* gene disruption strains to the wild-type strain from the same plate was calculated. Some plates had two trails of the wild-type or two trails of the *dipks14* gene disruption strain with one of the opposite strain. In this case, the wild-type and gene disruption strain zones of migration on the same plate were compared twice.

Slug phototaxis assay

10 microlitres of washed cells at 2×10^8 cells per mL were spotted on opposite sides of water-1% charcoal-1% agar plates and allowed to develop for 24 hrs in the dark with a point source of light equidistant from the spots. Slug migration was documented by inverting the plates and scanning

them on an HP scanner and images were inverted. For examination of slug size, observations were made at 16 hrs after the start of development on water-charcoal-agar plates using an Olympus compound microscope.

Image analysis

Images were taken with a digital camera and plates were scanned on an HP scanner. Images were transferred to the ImageJ software program. Image analysis was performed using the ImageJ software.

RESULTS

Disruption of the *Dictyostelium discoideum dipks14* gene

The DiPKS14 protein is predicted have eight catalytic domains on a single polypeptide and encodes a megasynthase ((Muthukumar and Manjunath 2020), Figure 1). The KS, AT, KR and ACP domains are called the core domains and are essential for gene function. We planned to disrupt the core domains KR and ACP to ensure the gene is non-functional. We first created a gene disruption or gene targeting vector. The *dipks14* gene disruption vector was constructed by subcloning two homologous regions from the *dipks14* gene into the pBS-BSR empty targeting vector. The regions of homology are referred to as the 5′arm and the 3′ arm of homology and were PCR amplified from the *dipks14* genomic region using the gene specific primers-pairs rsg1783-rsg1784 and rsg1785-rsg1786 for the 5′arm and the 3′arm of homology respectively. The 5′ arm of homology was subcloned into the NotI/XbaI restriction sites and the 3′ arm of homology was subcloned into the HinDIII/KpnI restriction sites. The resultant plasmid vector was designated *DiPKS14* KO Vector (Figure 2).

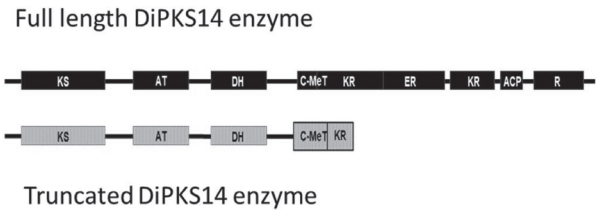

Fig. 1. Schematic of the protein domain organization of DiPKS14. The DiPKS14 predicted protein has eight domains organized on a single polypeptide. KS (ketosynthase), AT (acyltransferase), DH (dehydratase), C-Met (c-methyltransferase), KR (ketoreductase), ER (enoyl reductase), KR (ketoreductase), ACP (acyl transferase protein), R (reductase domain). Also depicted below is the possible predicted partial protein from a truncated transcript after disruption of the *dipks14* gene.

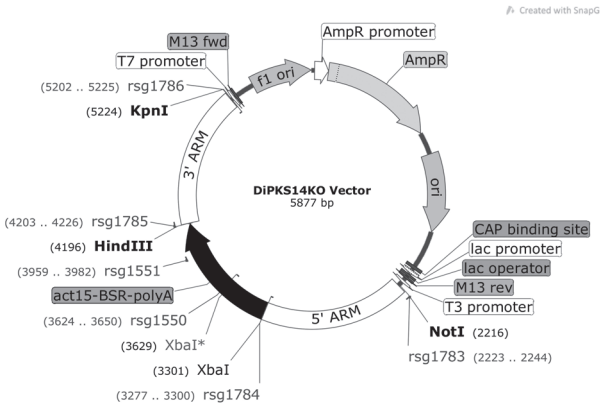

Fig. 2. Schematic of gene disruption vector: Two ~1kb regions homologous to parts of the *dipks14* gene (white boxes) were subcloned into the empty pBS-BSR gene disruption vector on either side of the BSR selection cassette (black box) to generate the *dipks14* gene disruption vector.

The targeting cassette consisting of the 5′and 3′ arm of homology and the BSR resistance cassette was introduced into wild-type *Dictyostelium discoideum* cells using electroporation. The *dipks14* gene disruption was achieved by swapping the Blasticidin resistance cassette or a selectable marker into the coding region of *dipks14* within the keto reductase or KR domain (Figure 3). This deleted 381bp of the region coding for the KR domain and

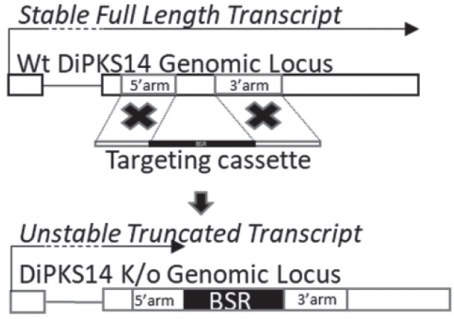

Fig. 3. Cartoon of the *dipks14* gene disruption strategy. White boxes represent the wild-type and the *dipks14*ko genomic. 5′arm and 3′arm are the regions of homology in the genomic locus and the targeting cassette. The line represents is the targeting cassette. Black box is the BSR antibiotic resistance selection marker. Arrows are the predicted transcripts from the wild-type and the *dipks14* gene knockout genomic locus.

disrupted the KR and the following ACP and R domains thus was expected to render the gene non-functional (Figure 1). The knockout strains (*dipks14*ko) were selected for by growing the cells in media with the antibiotic Blasticidin. PCR amplification was conducted using genomic DNA isolated from wild-type and *dipks14*ko strains and the genomic primers rsg1895 and rsg1896. This primer pair was expected to yield a 2918bp fragment from wild-type and a 3481bp fragment from the *dipks14*ko genomic templates. As expected we obtained a 2.9kb and a 3.5kb amplicon from the wild-type and the *dipks14*ko genomic locus respectively. This gel-shift accounted for the insertion of the 931bp long BSR selection cassette with the concomitant removal of the 381bp fragment of the *dipks14* gene encoding a part of the essential KR or ketoreductase domain of the predicted DiPKS14 enzyme (Figure 4).

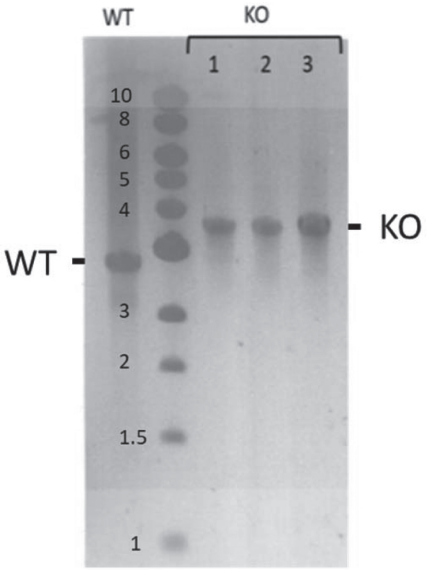

Fig. 4. PCR confirmation of the *dipks14* gene disruption. PCR amplification of a part of the genomic locus of the wild-type (WT) and the *dipks14* gene disruption strain (KO) using *dipks14* gene specific primers was conducted. The amplicons were separated by gel electrophoresis alongside the NEB 1kb DNA ladder. The amplicon from the dipks14 gene disruption displayed a gel-shift upwards as compared with the wild-type due to the insertion of the BSR selection cassette.

The *Dictyostelium discoideum Dipks14*KO strain has a slug migration defect

The slug stage of the *Dictyostelium discoideum* is motile and shows both non-directed (without external stimulus) and directed (with an external stimulus like heat-thermotaxis, chemicals-chemotaxis and light-phototaxis) movement. To test the effect of the loss of *dipks14* on *Dictyostelium discoideum* we first performed a non-directed movement assay. In the absence of a light source, slugs move radially outward from a crowded center, leaving behind a network of slime trails. These slime trails consist of slime, cells and other debris left behind from the rear guard region of the slug. This can be stained and observed with coomasie blue. The slime trails by 24 hours have an approximately circular area as determined by the quadruple measurements of diameter in the vertical, horizontal, left diagonal and right diagonal directions. We called this the zone of migration. The zone of migration in the *dipks14* gene disruption strains was approximately half the diameter of the wild-type as was the area (Figure 5). The ratio of the diameters of the zones of migration of *dipks14*ko to that of wild-type KO:WT was 0.574, SD = 0.089 (Table 1). The ratio of diameters of wild-type ZOM's between independent plates, WT:WT, was 1.0, SD = 0.17, and that of *dipks14*ko ZOM's between independent plates, KO:KO, was 1.0, SD = 0.12. The *dipks14*ko slugs do not seem to migrate out from the spot and no slime trails were observed. This suggests that DiPKS14 is required at the slug stage in the life cycle of *Dictyostelium* and might be essential for normal slug migration.

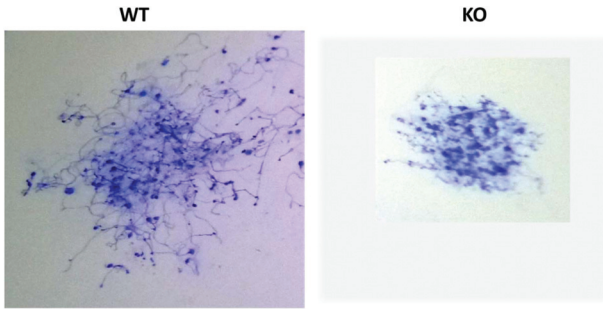

Fig. 5. Comparison of the results of the undirected slug migration assay between the wild-type (WT) and the *dipks14* gene disruption strain (KO). Cells were spotted at the center of a water-agar plate and allowed to develop in the dark for 24 hours. Slime trains were transferred to nitrocellulose membranes and stained with coomasie blue and documented by imaging using a camera.

Some *Dipks14*KO slugs are able to phototax towards light and are smaller than wild-type slugs

Directed phototactic behavioural phenotyping of the slug stage was also conducted. Cells spotted on non-nutrient agar, develop into slugs at 16 hrs post

Table 1. Comparison of the zone of migration of wild-type and dipks14 gene disrupted strain slugs.

Zone of Migration (ZOM)	Plate1		Plate 2		Plate 3			Plate 4		Plate 5		
in pixels	WT	KO	WT	KO	WT	KO	KO	WT	KO	WT	WT	KO
Vertical	137.333	77	110.667	76	174.667	73	92	138.67	109	164	178.667	78.006
Horizontal	138.667	76	137.333	78.667	168	78.667	82.667	144	85.333	146.667	141.333	92
Left Diagonal	120.68	73.539	124.451	81.377	188.562	82.967	88.624	137.64	89.572	143.307	181.019	86.183
Right Diagonal	143.307	81.082	145.193	88.624	139.536	79.521	79.218	135.77	101.823	169.706	177.248	71.653
Average Diameter of ZOM	**134.997**	**76.9053**	**129.411**	**81.167**	**167.691**	**78.539**	**85.6273**	**139.02**	**96.432**	**155.92**	**169.567**	**81.9605**
Ratio of Diameter KO:WT		0.56968		0.6272		0.4684	0.51062		0.69367			0.52566

Wild-type (WT) and *dipks14* gene disrupted (KO) cells were spotted on water-agar plates in 5 independent experiments and allowed to develop for 24 hours. Slime trails were approximately circular and this was called the zone of migration (ZOM). The diameter of each zone of migration was quantified four times in four different directions, vertical, horizontal, left diagonal and right diagonal and averaged to obtain the average diameter of each zone. The ratio of the diameter of the dipks14 gene disrupted and the wild-type strains (KO:WT) was calculated for slime trails on the same plate.

spotting. To determine the cause of the slug migration defect seen in *dipks14*ko strains, the slugs were observed more closely. Cells were spotted at the same cell density of $2x10^8$ cells per ml and allowed to develop for 16 hours with a point source of light. *dipks14*ko slugs were smaller in size and remained closer to the spot at 16 hours of development compared to the wild-type slugs which had migrated further out (Figure 6). Wild type slug length was an average of 1.2 mm whereas the *dipks14* gene disruption slugs were on an average 0.8mm long (Figure 7). 75% of the *dipks14*ko slugs were below 1.0mm in length whereas 75% of the wild-type slugs were above 1mm long. Since it was obvious by observation that the *dipks14*ko slugs were smaller than the wild-type slugs, a one tailed t-test was conducted to determine the t-value and the significance p-value. 12 *dipks14*ko slugs (M = 886.63, SD = 277.64) compared to the 16 control wild type slugs (M = 1226.14, SD = 296.69) had significantly (p

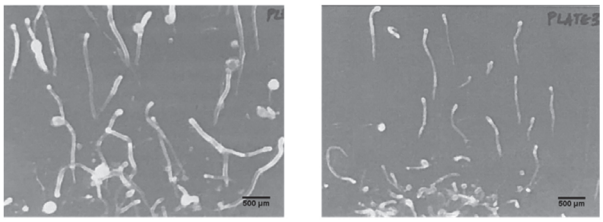

Fig. 6. A comparison of the slug morphology of the wild-type (WT) and the *dipks14* gene disrupted (KO) strains. Wild-type and *dispk14* gene disrupted cells were spotted on charcoal agar plates and allowed to develop for 16 hours. The plates were imaged at 10X magnification on an Olympus microscope just as the slugs started to migrate.

< 0.05) smaller length (t value = -3.0785, $p = 0.00243$). This suggests that DiPKS14 might be required for both slug size and slug migration.

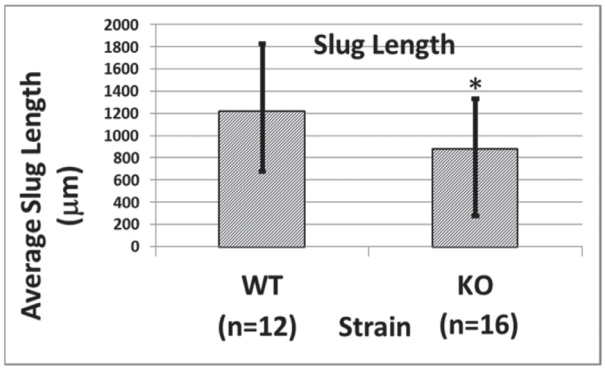

Fig. 7. Quantification of slug length. Slugs from wild-type (WT) and the *dipks14* gene disrupted strain (KO) were imaged under the microscope and the slug length quantified using ImageJ. Slug lengths were statistically analysed by a one-tailed t- test. * indicates that the *dipks14* gene disrupted slugs were significantly smaller than the wild-type slugs. $t = -3.0$ and $p = 0.002$.

Disruption of the *dipks14* gene results in fewer migrating slugs

To determine if the disruption of DiPKS14 had an effect on the number of migrating slugs, 10 microlitres of cells of each strain were plated in quadruplicate at a density of $2X10^8$ cells per mL and allowed to develop in the dark in the presence of a point source of light. The number of migrating slugs per strain was counted and the average number of migrating slugs per strain was calculated. On an average wild-type cells produced more migrating slugs (~80 slugs per $2X10^6$ cells) as compared to the *dipks14*ko strain (~50 slugs per $2X10^6$ cells) (Figure 8). Our results show that there are significantly fewer migratory slugs ($t = 11.97$, $p = 0.00001$) in the *dipks14*ko strain (n = 189, M = 47.25, SD = 3.77) as compared with the wild-type strain (n = 331, M = 82.75, SD = 4.57). This suggests that DiPKS14 might

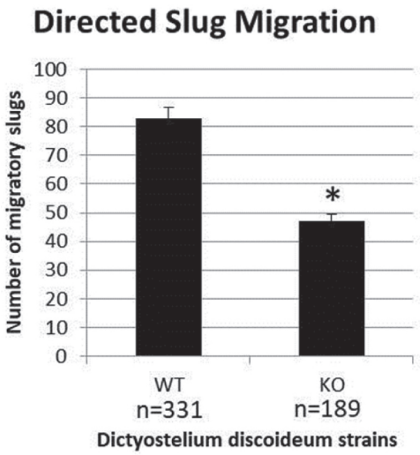

Fig. 8. Comparison of the number of migrating slugs in wild-type versus the dipks14 gene disruption strain. Wild-type (WT) and *dispk14* gene disruption (KO) cells were allowed to develop in the dark on charcoal water agar plates with a point source of light for 24 hours and the number of migrating slugs in each strain was counted. * indicates that the number of migrating slugs in the dipks14 gene disruption strain was significantly lower than the in wild-type.

be required for both slug size and slug migration. The loss of DiPKS14 either results in fewer slugs or that of the total number of slugs formed only the smaller slugs are able to migrate. In fact it observed that the slugs in the center were of normal size but did not move out suggesting that DiPKS14 might be required for motility of normal sized slugs.

*dipks14*ko gene disruption strain migrating slugs are still able to orient and move towards light

To test whether the disruption of DiPKS14 affects phototaxis behavior of the slugs, 10 µL of wild-type and Knockout cells were spotted at equal density on opposite sides of the same water-charcoal-agar plate. A point source of light equidistant from both spots was provided as shown in the figure. The cells were allowed to develop for 24 hours till fruiting bodies had formed. In the presence of a point source of light, slugs that form at 16 hours start to move towards the light. Observations at 24 hours revealed that *dipks14*ko slugs had not migrated as far as wild-type slugs although they still aligned themselves towards the light source and exhibited some migration (Figure 9). This suggests that disruption of DiPKS14 does not affect that component of the organized prestalk region that is required for phototactic turning but might be required for slug migration and slug size alone.

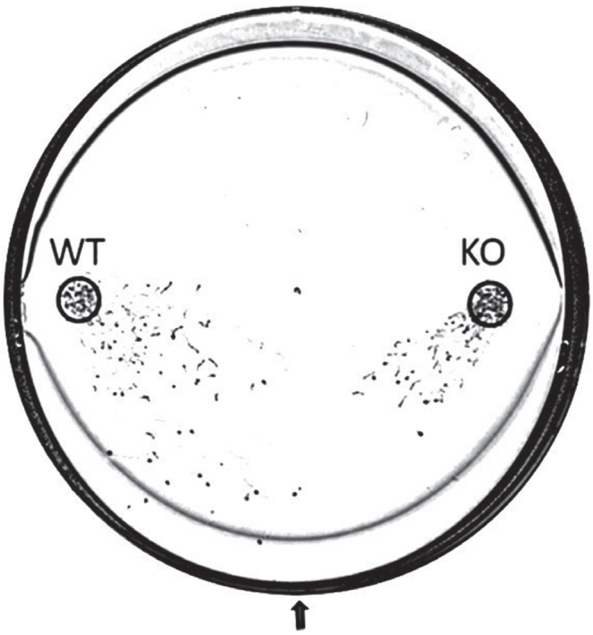

Fig. 9. Comparison of the phtototactic behaviour of wild-type (WT) and the *dipks14* gene disrupted strain (KO). Circles indicate the initial area where the cells were spotted on a 90 mm charcoal agar plate. The black arrow indicates a point source of light in an other-wise darkened plate. The tiny scattered dots are the point at which slugs stopped migrating and formed a fruiting body. Cells were allowed to develop for 24 hours at 22 °C prior to imaging.

DISCUSSION AND CONCLUSION

Polyketides are thought to be secreted toxic by-products of the secondary lipid metabolism, however recent evidence points to the *in vivo* use of Polyketides as building blocks and as a means of communication. In angiosperms PKS enzymes present in the endoplasmic reticulum synthesize Polyketides which are then secreted (Lallemand *et al.,* 2013). Genetic approaches have identified that PKS genes are essential for pollen viability, formation of Sporopollenin and integrity of the pollen wall (Kim *et al.,* 2010). PKSs are required for the synthesis of unusual lipids in the mycobacterial cell wall (Trivedi *et al.,* 2005; Herbst *et al.,* 2016; Gokhale, 2001) and for *Mycobacterial* sliding motility (Vats *et al.,* 2012). In *Azotobacter vinelandii* PKS genes are required for formation of the outer resistant coat of the dormant cyst (Funa *et al.,* 2006). They are required for the fruiting body morphology in the filamentous ascomycetes *Sordaria macroscopa* (Funa *et al.,* 2006). In Raspberries the characteristic

fragrance of the fruit that aids in seed dispersal is due to the presence of a polyketide p-hydroxyphenylbutan-2-1 (Borejsza-Wysocki and Hrazdina, 1996).

In *Dictyostelium discoideum,* disruption of DiPKS14 results in smaller, fewer slugs that do not migrate as far as the wild-type slugs. In the wild, slug migration aids in spore dispersal. Slugs migrate upward through the soil by phototaxis, come to the surface and set up a fruiting body, thus ensuring adequate spore dispersal. Speed of slug migration is directly proportional to slug size, length and age (Inouye and Takeuchi, 1979). On water-agar plates wild-type *Dictyostelium discoideum* slugs move out of a crowded area and set up individual fruiting bodies further away from each other. This is achieved through communication between slugs via secreted factors (Fisher et al. 1981) and gives each spore an equal chance of finding favorable conditions to germinate and thrive. Our data suggests that the smaller slug size and deficient migration seen in the *dipks14* gene knockout strains would be deficient in spore dispersal in the wild.

The many PKS genes in *Dictyostelium discoideum* are highly homologous and show 45% to 95% similarity. To determine whether *dipks14* plays a unique role in the life cycle of *Dictyostelium discoideum* a phenotypic analysis of the slug stage of *dipks14* gene knockout strain was conducted. This report shows that in *Dictyostelium discoideum* DiPKS14 function correlates with slug formation, slug size and their ability to migrate for long distances towards light. The disruption of the *dipks14* gene prevents slugs from moving away from each other, resulting in closely spaced fruiting bodies. This suggests that in the wild-type DiPKS14 might be required for maintenance of distance between multicellular bodies and consequently for efficient spore dispersal. Our data suggests that the Type I PKS gene DiPKS14 plays an important and unique role in the life cycle of *Dictyostelium discoideum*.

ACKNOWLEDGEMENT

All the molecular and developmental biology experiments were conducted in Dr.Rajesh S. Gokhale's laboratory at the National Institute of Immunology, New Delhi, 110067. We thank Prof. Shweta Saran (SLS, JNU) for the wild-type AX2 strain. Dr. Om Deshmukh (Director, Tildehat, Bengaluru) and members of the G.V. Soni Laboratory (Raman Research Institute, Bengaluru) for helpful discussions on statistical and image analysis tools.

REFERENCES

Araki, T. and Saito, T. 2019. Small molecules and cell differentiation in *Dictyostelium discoideum*. *International Journal of Developmental Biology*. 63(9–10): 429–483.

Austin, M.B., Saito, T., Bowman, M.E., Haydock, S., Kato, A., Moore, B.S., Kay, R.R. and Noel, J.P. 2006. "Biosynthesis of *Dictyostelium discoideum* differentiation-inducing factor by a hybrid type I fatty acid–type III polyketide synthase. *Nature Chemical Biology*. 2(9) : 494–502. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16906151 [Accessed June 26, 2017].

Bonner, J. 2009. *The Social Amoeba. The Biology of Cellular Slime Molds*. Princeton University Press.

Bonner, J.T. 1952. The Pattern of Differentiation in Amoeboid Slime Molds. *The American Naturalist* 86(827) : 79–89. Available at: http://www.journals.uchicago.edu/doi/10.1086/281707 [Accessed June 13, 2017].

Borejsza-Wysocki, W. and Hrazdina, G. 1996. Aromatic Polyketide Synthases (Purification, Characterization, and Antibody Development to Benzalacetone Synthase from Raspberry Fruits). *Plant Physiology* 110(3). Available at: http://www.plantphysiol.org/content/110/3/791.short?cited-by=yesl110/3/791 [Accessed June 26, 2017].

Chisholm, R.L. and Firtel, R.A. 2004. Insights into morphogenesis from a simple developmental system." *Nature Reviews Molecular Cell Biology*. 5 (7) : 531–541. Available at: http://www.nature.com/doifinder/10.1038/nrm1427.

Fisher, P.R., Smith, E. and Williams, K.L. 1981. An extracellular chemical signal controlling phototactic behavior by D. discoideum slugs. *Cell*. 23(3) : 799–807. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7226230 [Accessed June 13, 2017].

Fuchs, M., Jones, M.K. and Williams, K.L. 1993. Characterisation of an epithelium-like layer of cells in the multicellular *Dictyostelium discoideum* slug." *Journal of Cell Science*. 105 (1) : 243. Available at: http://jcs.biologists.org/content/105/1/243.short.

Funa, N., Ozawa, H., Hirata, A. and Horinouchi, S. 2006. "Phenolic lipid synthesis by type III polyketide synthases is essential for cyst formation in Azotobacter vinelandii. *Proceedings of the National Academy of Sciences of the United States of America* 103(16) : 6356–6361. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16597676 [Accessed March 22, 2019].

Ghosh, R., Chhabra, A., Phatale, P.A., Samrat, S.K., Sharma, J., Gosain, A., Mohanty, D., Saran, S. and Gokhale, R.S. 2008. Dissecting the Functional Role

of Polyketide Synthases in *Dictyostelium discoideum* Biosynthesis of the Differentiation Regulating Factor 4-methyl-5-pentylbenzene-1,3-DIOL * % S." Available at: http://www.jbc.org/content/283/17/11348.full.pdf?with-ds=yes [Accessed April 29, 2017].

Gokhale, R.S. 2001. *Biochemistry of Polyketide Synthases*.

Gokhale, R.S., Sankaranarayanan, R. and Mohanty, D. 2007. Versatility of polyketide synthases in generating metabolic diversity." *Current Opinion in Structural Biology*. 17(6) : 736–743.

Herbst, D.A., Jakob, R.P., Zähringer, F. and Maier, T. 2016. Mycocerosic acid synthase exemplifies the architecture of reducing polyketide synthases. *Nature* 531(7595) : 533–537. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26976449 [Accessed November 1, 2019].

Herbst, D.A., Townsend, C.A. and Maier, T. 2018. The architectures of iterative type I PKS and FAS." *Natural Product Reports*. 35(10) : 1046–1069.

Inouye, K. and Takeuchi, I. 1979. Analytical studies on migrating movement of the pseudo-plasmodium of *Dictyostelium discoideum*. *Protoplasma*. 99(4) : 289–304.

Kay, R.R., Dhokia, B. and Jermyn, K.A. 1983. Purification of stalkcellinducing morphogens from *Dictyostelium discoideum*. *European Journal of Biochemistry*. 136 (1) : 51–56.

Kim, S.S., E. Grienenberger, B. Lallemand, C.C. Colpitts, S.Y. Kim, C. de A. Souza, P. Geoffroy, D. Heintz, D. Krahn, M. Kaiser, E. Kombrink, T. Heitz, D.-Y. Suh, M. Legrand, and C.J. Douglas. 2010. "*LAP6/POLYKETIDE SYNTHASE A* and *LAP5/POLYKETIDE SYNTHASE B* Encode Hydroxyalkyl á-Pyrone Synthases Required for Pollen Development and Sporopollenin Biosynthesis in *Arabidopsis thaliana*." *The Plant Cell* 22(12):4045–4066. Available at: http://www.plantcell.org/lookup/doi/10.1105/tpc.110.080028.

Lallemand, B., Erhardt, M., Heitz, T. and Legrand, M. 2013. Sporopollenin Biosynthetic Enzymes Interact and Constitute a Metabolon Localized to the Endoplasmic Reticulum of Tapetum Cells. *Plant Physiology.* 162(2) : 616–625. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23632852 [Accessed September 30, 2018].

Manocha, A. 2011. Understanding Biochemical Mechanism Involved in Development of *Dictyostelium discoideum*. *PhD Thesis,Submitted to Delhi University*.

Muthukumar, L. and Manjunath, K. 2020. The *Dictyostelium discoideum dipks14*: a membrane anchored highly reducing iterative polyketide synthase similar to ones found in pathogenic fungi and mycobacteria. *Asian Jr. of Microbiol. Biotech. Env. Sc.* : 120–131. Available at: http://www.envirobiotechjournals.com/article_ abstract.php?aid=10368&iid=299&jid=1 [Accessed June 16, 2020].

Nair, D.R., Ghosh, R., Manocha, A., Mohanty, D., Saran, S. and Gokhale, R.S. 2011. Two functionally distinctive phosphopantetheinyl transferases from amoeba *Dictyostelium Discoideum*. *PLoS ONE*. 6(9) : 1–10.

Narita, T.B., Chen, Z. H., Schaap, P. T. Saito, and Bloomfield, G. 2014. The Hybrid Type Polyketide Synthase Steely a is Required for cAMP Signalling in Early Dictyostelium Development. *PLoS ONE* 9(9). Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4164351/pdf/pone.0106634.pdf [Accessed May 23, 2017].

Raper, K.B. 1935. *Dictyostelium Discoideum*, A New Species of Slime Mold from Decaying Forest Leaves. Available at: https://naldc.nal.usda.gov/download/IND43968646/PDF [Accessed September 30, 2018].

Staunton, J. and Weissman, K.J. 2001. Polyketide biosynthesis: a millennium review. *Natural Product reports* 18(4) : 380–416. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11548049 [Accessed June 13, 2017].

Trivedi, O.A., Arora, P., Vats, A., Ansari, M.Z., Tickoo, R., Sridharan, V., Mohanty, D. and Gokhale, R.S. 2005. Dissecting the Mechanism and Assembly of a Complex Virulence Mycobacterial Lipid. *Molecular Cell*. 17 (5) : 631–643. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1097276505010865 [Accessed June 26, 2017].

Vats, ArchanaVats, A. 2012. Retrobiosynthetic A.D. the B.P. and the S. of the A.C. of M.G.* % S. doi: 10. 1074/jbc. M. 384966., A. Kumar Singh ^, R. Mukherjee, T. Chopra, M. Sudhan Ravindran, D. Mohanty, D. Chatterji, Reyrat, J. M. and Gokhale, R.S. 2012. Retrobiosynthetic Approach Delineates the Biosynthetic Pathway and the Structure of the Acyl Chain of Mycobacterial Glycopeptidolipids * % S." Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3436312/pdf/zbc30677.pdf [Accessed January 7, 2018].

Williams, J.G. 2010. Dictyostelium finds new roles to model. *Genetics*. 185 (3) : 717–726.

Zucko, J., Skunca, N., Curk, T., Zupan, B., Long, P.F., Cullum, J., Kessin, R.H. and Hranueli, D. 2007. Polyketide synthase genes and the natural products potential of *Dictyostelium discoideum*. *Bioinformatics* 23(19):2543–2549. Available at: https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btm381 [Accessed June 26, 2017].