Development and Characterization of Expression Vectors for the Riboflavin Overproducing Fungus *Eremothecium ashbyii* using *Ashbya gossypii* Genes

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Abstract

*Eremothecium ashbyii* is a filamentous hemi-ascomycete fungus and a natural overproducer of riboflavin. The present study was undertaken to characterize the molecular tools constructed for the genetic manipulation of this organism based on plasmids constructed for the related organisms *A. gossypii* and *S. cerevisiae* using two candidate genes. The candidate gene, *SPR3* homolog of *S. cerevisiae*, is known to play a role in cytokinesis in *S. cerevisiae*. This gene was chosen to aid in future studies on the regulation of septation and its role in the excretion of riboflavin in *E. ashbyii*, as yeast cytokinesis is analogous to the septation of filamentous fungi. The second candidate gene was the *S. cerevisiae RAD14* homolog, which is known to play a key role in the nucleotide excision repair pathway. Reporter plasmids, constructed previously in a preliminary study with the *AgSPR3*-like gene and the *AgRAD14*-like gene fused to the *LacZ* reporter gene, were used in this study. These plasmids were characterized by sequencing followed by homology searches. While the former revealed homology to the *S. cerevisiae* septin protein family, *SPR3* gene, and the *Neurospora crassa* CDC12 gene involved in cell cycle regulation, the latter showed homology to the *S. cerevisiae* HOG1 gene involved in the osmotic stress response.

Keywords – Cytokinesis – *HOG1* – NER pathway – Septation – *SPR3*

Introduction

*Eremothecium ashbyii* is a filamentous hemi-ascomycete fungus with a genome similar to the yeast *Saccharomyces cerevisiae*. It is known for the natural overproduction of riboflavin (Zhang et al. 2021), thus making it industrially important. Riboflavin, also known as vitamin B₂, is important for cells. It forms the main component of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which function as coenzymes for many oxidoreductases (Zhang et al. 2021). It is an important ingredient in multivitamin formulations and is also used as a food additive and in the formulation of animal feed supplements.
Morphological studies of *E. ashbyii* show the presence of slimy colonies. The mycelium is found to be coenocytic at the earliest stage, which eventually divides into plurinucleate segments of unequal length by forming callose plugs. Swelling of the segments leads to the division of nuclei, causing condensation of the sporoplasm around the nuclei. Ascospores, usually 8–16 per ascus, are hyaline and smooth. Sporulation is correlated with riboflavin production (Pujari & Chandra 2001). Non-sporulating *E. ashbyii* produces less riboflavin (Zhang et al. 2021). It has been shown that *E. ashbyii* accumulates lipids in its mycelia before riboflavin overproduction (Vijayalakshmi et al. 2010). The amount of lipid accumulated in mycelia decreases as riboflavin production increases. Monitoring of the lipid accumulation was done with the fluorescent probe Nile blue (Vijayalakshmi et al. 2003). The lipid accumulation was correlated with extracellular lipase activity and also with the production of riboflavin (Vijayalakshmi et al. 2010). Riboflavin is transported out of the overproducer’s cytoplasm rather than being stored in the cell.

In *E. ashbyii*, one of the mechanisms of countering riboflavin-induced toxicity is through the modulation of membrane fluidity by incorporating a large percentage of unsaturated fatty acids, and notably oleic acids, in the membrane phospholipids (Vijayalakshmi et al. 2010). Preliminary studies have shown that morphological changes are produced as a first line of defense (Sampath & Vijayalakshmi 2018). Another line of adaptation to stress is the change in membrane fluidity as a result of alterations in the composition of the membrane phospholipids (Vijayalakshmi et al. 2010). Regarding riboflavin overproduction by *E. ashbyii*, a question of relevance to be addressed is whether changes in membrane fluidity alone are enough to allow the riboflavin to be excreted. Cellular processes such as vesicle trafficking, cytoskeletal organization, and regulation are vital to riboflavin production and excretion. Septation is an important process that plays a role in the organism’s development. Hence, the proteins involved in septation and cytoskeletal organization are good candidates for researching the role of the cytoskeleton and septation in riboflavin production and excretion in *E. ashbyii*. The SPR3 gene is known to play a role in cytokinesis in *S. cerevisiae* (Alexander & Michelle 2019). The cytokinesis process in yeast is analogous to the process of septation in filamentous fungi. Hence, this *SPR3* gene homolog was chosen to aid future studies on the regulation of septation and its role in riboflavin excretion in *E. ashbyii*.

Excess endogenous riboflavin is known to cause photo-induced damage similar to UV damage, causing DNA, RNA, and protein damage, whereas exogenous riboflavin protects the spores (Silva et al. 2019; Sugimoto et al. 2010). Organisms subjected to UV treatment start producing riboflavin at an early stage, accounting for the importance of maintaining their genomic integrity. Though a change in membrane fluidity and composition of membrane phospholipids has been implicated in countering riboflavin toxicity (Vijayalakshmi et al. 2010), the above findings point to the possible existence of another pathway triggered by UV exposure. UV-induced damage is mostly repaired by the RAD proteins of the Nucleotide Excision Repair (NER) mechanism (Perego et al. 2000) and is probably active during the overproduction of riboflavin. The genome of *E. ashbyii* is similar to that of *S. cerevisiae* (Prillinger et al. 1997). The NER pathway has been characterized in detail in *S. cerevisiae*, in which several proteins like RAD1, RAD10, and RAD14 have been shown to play a role. The NER may probably be functioning in *E. ashbyii* to combat riboflavin toxicity, thus maintaining the genome stability of the organism. Hence, the *S. cerevisiae RAD 14* homologue was chosen with a view to aiding future studies on the role of the NER pathway in combating riboflavin-induced stress in *E. ashbyii*.

Molecular studies have been successfully undertaken in *A. gossypii*, and many vectors have been constructed for use in *A. gossypii* (Wright & Philippsen 1991, Steiner et al. 1995, Wendland et al. 2000). Additionally, the genome of *A. gossypii* has been completely sequenced (Dietrich et al. 2004), making genetic studies feasible in this organism. However, no genomic studies have been carried out so far on *E. ashbyii*. In another study, vectors constructed in *A. gossypii* were used to successfully transform *E. ashbyii* (Manoj & Vijayalakshmi, unpublished results). Hence, in this study, the vectors were constructed using *A. gossypii* genes to aid in future studies on *E. ashbyii*.  

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Materials and Methods:

Organism and culture conditions

Eremothecium ashbyii MTCC 366 (also known as NRRL 1363) was cultured on Potato Dextrose Agar slants and plates (PDA g/l, Potato infusion-200, Dextrose-20, Agar-20 pH 5.6± 0.2) and cultures were maintained at 25 °C. Subculturing was done at 15-day intervals on PDA slants to maintain the culture.

Chemicals

All chemicals used were of analytical grade from local sources.

DNA manipulations

All DNA manipulations were carried out according to Sambrook & Russell (2001) using the Escherichia coli DH5-α strain as the host (Hanahan 1983). Before cloning, the sequences of the PCR-amplified fragments were verified by sequencing. Sequencing was done by Eurofins (Bangalore, India). The proper orientation of insertion was verified by restriction analysis. Competent cells were prepared, and the ligated DNA mixture was mixed with competent cells and transformed by the heat shock method. Transformed colonies were selected by the blue/white screening method (Green & Sambrook 2012).

Plasmids and constructs

Plasmids used in the present study are listed in Table 1. PCR primers used are listed in Table 2.

Plasmid isolation

Plasmid isolation was carried out using the alkaline lysis method. Single transformed colonies were grown overnight in 10 ml of LB broth containing ampicillin (50 μg/ml). A 1.5 ml of LB broth was centrifuged at 10,000 rpm for 2 min at 4 °C. A 100 μl of the re-suspension buffer (50 mM glucose, 10 mM EDTA, 25 mM TrisHCl, pH 8.0) was added to the pellet and mixed properly.

A white precipitate was formed, indicating the presence of chromosomal DNA when 200 μl of lysis solution (0.2N NaOH, 1% SDS) was added to the above mixture. Finally, 150 μl of neutralizing solution (of composition per 100 ml: 60 ml of 5M potassium acetate, 11.5 ml of glacial acetic acid and 28.5 ml of deionized water) was added to the solution. After the addition, centrifugation was done at 10,000 rpm for 10 minutes at 4 °C. To the supernatant, an equal amount of phenol: chloroform: isoamyl alcohol (25:24:1) was added. The top aqueous layer was collected after centrifuging at 10,000 rpm for 2 minutes. Then, 1 ml of 100% ethanol was added to the top aqueous layer and collected. Centrifugation was done at 10,000 rpm for 5 minutes at -4 °C, and the pellet was collected. To the pellet, 1 ml of ice-cold 70% ethanol was added and centrifuged at 10,000 rpm for 2 minutes. The collected pellet was air-dried and re-suspended with 100 μl TE buffer (Sambrook & Russell 2001).

Table 1. List of Plasmids used in the present study and their properties

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Properties</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid BB</td>
<td>Amp', Gen'A derivative of pSV 2 in which the RIB 3 gene is excluded and restriction sites for integration of RAD 14 are included</td>
<td>Sampath &amp; Vijayalakshmi (2009), unpublished thesis work.</td>
</tr>
<tr>
<td>Plasmid D</td>
<td>Amp', Gen'A derivative of pSV 2 and contains the lacZ reporter gene from pTS 24 (Schlösser, 2007), under the control of the SPR 3 promoter</td>
<td>Vinod &amp; Vijayalakshmi (2009), unpublished thesis work.</td>
</tr>
</tbody>
</table>
**Restriction digestion**

Isolated plasmid DNA was subjected to restriction digestion as follows: 2 µl of each plasmid DNA was taken separately, and 1 µl of the 10X tango buffer (ThermoFisher) was added to each sample. To this mixture, 2 µl of restriction enzyme *BamH1* (ThermoFisher) was added, and the samples were incubated in the water bath at 37 °C overnight. Later, the reaction mix was electrophoresed on a 1% agarose gel.

Table 2. List of primers used in PCR

<table>
<thead>
<tr>
<th>Primer Sequence (5′-3′)</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sam1</strong> <em>RAD 14</em> (Promoter + ORF)</td>
<td>A. <em>gossypii</em> genomic DNA</td>
</tr>
<tr>
<td>Forward: ATG CTG <strong>GGG CCC</strong> ATG GCG CTG ACC GCA GAG</td>
<td></td>
</tr>
<tr>
<td>Reverse: ATG CTG <strong>GCT AGC</strong> CTA CAA CAT TAT TTC TAT TTC TAT C</td>
<td></td>
</tr>
<tr>
<td><strong>Sam2</strong> <em>RAD 14</em> (Promoter)</td>
<td>A. <em>gossypii</em> genomic DNA</td>
</tr>
<tr>
<td>Forward: CAT <strong>GGG CCC</strong> TTA CTA GCG TGA CCC CCG GG</td>
<td></td>
</tr>
<tr>
<td>Reverse: ATC <strong>GCT AGC</strong> GAA CAG ACA GGA GCC TAT GTA G</td>
<td></td>
</tr>
<tr>
<td><strong>Vin1 – SPR3</strong> (Promoter + ORF)</td>
<td>A. <em>gossypii</em> genomic DNA</td>
</tr>
<tr>
<td>Forward: ATT <strong>CGG GCC</strong> CCG TCA GAG TGC ACA AGC TCG</td>
<td></td>
</tr>
<tr>
<td>Reverse: ATT <strong>CGC TAG</strong> CCT TGC TGC TCA TGC TGC CC</td>
<td></td>
</tr>
<tr>
<td><strong>Vin2 – SPR3</strong> (Promoter)</td>
<td>A. <em>gossypii</em> genomic DNA</td>
</tr>
<tr>
<td>Forward: ACT <strong>TGG GCC</strong> CCG TCA GAG TGC ACA AGC TCG</td>
<td></td>
</tr>
<tr>
<td>Reverse: ACT <strong>TGC TAG</strong> CCT CCG TCC TTT TTA TTC TGT TTG</td>
<td></td>
</tr>
<tr>
<td>Sequence for <em>Apa</em> I - GGG CCC</td>
<td></td>
</tr>
<tr>
<td>Sequence for <em>Nhe</em> I - GCT AGC</td>
<td></td>
</tr>
</tbody>
</table>

**Result**

The plasmids were constructed using *A. gossypii* genes. Homologous recombination has been reported as the main mechanism for DNA integration in *A. gossypii* (Steiner et al. 1995). Since *A. gossypii* vectors bearing *A. gossypii* genes have been experimentally proven to successfully transform *E. ashbyii* (Fig. 1) (Manoj & Vijayalakshmi, unpublished results), it is quite possible that homologous recombination may prove to be the main mechanism for transformation in *E. ashbyii* as well. Therefore, we used *A. gossypii* genes to construct vectors for *E. ashbyii*.

The plasmid D is a derivative of the original integrative plasmid pSV 2 (Vijayalakshmi et al. 2003), which contains the GFP gene under the control of the *RIB 3* promoter and also specific loci for the targeted integration of the plasmid into the *A. gossypii* genome. In this plasmid, the *RIB 3* promoter was replaced by the *A. gossypii SPR 3* gene/promoter homolog, the Lac Z gene replaced the GFP gene, and the whole fragment was cloned into the *Bgl* II restriction site of plasmid pSV 2. The cloning was verified by linearizing the plasmids using *BamH1* digestion.
Plate showing *E. ashbyii* transformed with RIB3p GFP bearing Plasmid (pSV-2)

*RIB3p* GFP Expressing cells in Transformant (right panel) Control: wild type

*E. ashbyii* (left panel)

**Fig. 1** – Preliminary studies on *E. ashbyii* transformation using the pSV-2 vector constructed using *A. gossypii* genes. The image on the top is the plate on which *E. ashbyii* is transformed with a pSV-2 vector constructed using *A. gossypii* genes, showing the successful transformation. The image below is the fluorescent microphotograph of the *E. ashbyii* filaments expressing *A. gossypii RIB3p* GFP (pSV-2) (Bar, 100 μm) (right panel), thus showing that *A. gossypii* vectors are functional in *E. ashbyii*. The left panel is wild type of *E. ashbyii* with no GFP expression, indicated by the dark background.

**Characterisation of Plasmid D**

The plasmid D sequencing data was subjected to bioinformatics searches using the KEGG database, Unit Prot and SGD for homology. The nucleotide sequence of both the transcribed and the flanking regions of *SPR3* revealed a 640-bp open reading frame (Fig. 2) capable of encoding a 210-aa polypeptide chain (Fig. 3). The data showed a significant sequence homology with genes belonging to the septin family.

**The gene sequence of Plasmid D harbouring homologue to SPR3 gene**

ACGGACTGGCGAGTGGTTCCGTATTAGGAAATGCGATAATGAAATGCGCCCTGCGTTGAGGCCTGGTTTA
AGTATTCTCTTTATG
ACTGTTCTTAACCTTTAGGAACACTAGGGGCTTTACGCTTTCGCAAAATGAAACCAACCAGTGGG
TAGATGGGCAAACAA
GCCTCTTAAGATTGAGATAATGCTTCTGGGAGGGTTCCGTTAGGATCATTGCTGGAATCCCAGTATG
GCACGAGAATTGGGT
Amino acid sequence analyses in the ProSite database showed the presence of the highly conserved P-loop region (Fig. 2). The presence of the P-loop (ATP/GTP binding site motif) is a characteristic feature of many septin family proteins. The analysis also revealed the presence of two leucine zipper domains in the Ag SPR3p (Fig. 3). The leucine zipper pattern is present in many gene regulatory proteins such as C/EBP, CREB, C-myc, etc. As Ag SPR3p belongs to the septin family, which has protein regulatory functions, it is important to probe if Ag SPR3p is a nuclear DNA-binding protein. If it turns out to be a DNA-binding protein, Ag SPR3p’s regulatory role may not be limited to the protein level alone.

Comparison of the predicted amino acid sequence using the UniProtKB

The database revealed a significant sequence homology with genes belonging to the septin family (Fig. 4). The AgSPR3-like protein has a maximum percentage of sequence identity (100% similarity with 1st score bit and 49% with 2nd score bit) with the Kluyveromyces lactis protein KLLA0B08129g, which belongs to the septin family of proteins (Figs. 5 & 6), a high degree (almost 98%) of homology to A. gossypii (strain ATCC 10895) as shown in Fig. 4. In contrast to our point of interest, 78% homology has been seen with septin-type G protein of Eremeothecium cymbalariae (Fig. 7) and least with S. cerevisiae SPR3p of 47%, (Fig. 9). The highest match of 73% with Kluyveromyces marxianus (strain DMKU3-1042/BCC 29191/NBRC 104275) protein has been found which is known as cell division protein 3 (Fig. 10) and the least match of 38% with CDC12p of Neurospora crassa, a zinc finger protein, which is involved in cell division (Fig. 11). This indicates that the Ag SPR3 gene could have a potential role in the process of septation and can be probed further for such a role as well as a regulatory role on account of its homology to zinc finger proteins.
Fig. 3 – Predicted polypeptide sequence of Ag SPR3p. The P-loop region is highlighted in blue, and the two leucine zipper patterns are highlighted in red.

\[
\text{MSENQGTTSGGLHEDDSQNYTN} \text{SETISMSADQRAVSQAQTLSQSETAASSHSGQQATLDPEEHHTSLGY}
\]
\[
\text{EVERKADDEQDASSNNMDQLLEGVYFQHD} \text{DTETTNAHDKTRKLVEKKPISDRYRVGIEC} \text{LPLQREFV}
\]
\[
\text{AKKGGHFTVMVV} \text{GQTGLGKTTFVNTLFRTSLLPSVWDTLEG} \text{NKPNVQFKKTTRIRHQA} \text{LIEEKNIKLKLTVIDTPGF}
\]
\[
\text{DGDNANSFAWSPI} \text{YSISDEQFRSYIFQEEQPDRRLSDNRHI} \text{CCLYLFLNPSNKGISPL} \text{LIEAMMQEISK}
\]
\[
\text{RVNLIPVIAKADSLGTQSIAAFKEDVRRIANAGIRCAF} \text{FLDES} \text{SECSVIRDSPYALVCCDSYVQPKPNGEK}
\]
\[
\text{VRGRKYKGIGA} \text{EVENP} \text{KHDSCQLRDIL} \text{MSKNMVDLV} \text{VSSEKYETCRSHMLMTRINQAKD} \text{GLAAETSE}
\]
\[
\text{DNLILKNMNYESPDANGMLNYKCYIQYNKYQ} \text{YMHELIEWSPEFIHKQWEAKKRLNEIAH} \text{SEETKFTWKRA}
\]
\[
\text{LMFKHTN(LDSEIED(LHNVRKLN)} \text{QIDCEEL)QSEIIQLQTGGLGMSSKRMSKHDLLQ}
\]

**ATP/GTP-binding site motif A (P-loop)** 153 – 160: GqtglGKT

**LEUCINE ZIPPER Pattern** 500 - 521: LmfkhtnLdseiedLhnrvknL

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Fig. 4 – Comparison of predicted amino acid sequences for homology studies with the septin family.
Fig. 4 – Continued.

Fig. 5 – Sequence identity of Ag SPR3p with K. lactis KLLA0B08129g using UniProtKB: Identical matches are shown in the figure.
Fig. 5 – Continued.

### Fig. 6 – CAoGD database showing KLLA0B08129g having similarities with *S. cerevisiae* SPR3 and belongs to septin family protein with P-loop GTPase.
Fig. 7 – Sequence identity of Ag SPR3p with Eremeothecium cymbalariae showing 78% homology with Septin-type G domain-containing protein.
Fig. 7 – Continued.

Fig. 8 – Sequence identity of Ag SPR3p with Saccharomyces ludwigii showing 51% homology with its sporulation-regulated protein 3 (SPR3).
Fig. 8 – Continued.

Fig. 9 – Sequence identity of Ag SPR3p with S. cerevisiae SPR3p using UniProtKB: Identical matches are shown in the figure.
**Fig. 9** – Continued.

**Fig. 10** – Sequence identity match of Ag SPR3p with cell division control protein 3 of *Kluyveromyces marxianus* using UniProtKB.
Fig. 10 – Continued.
Fig. 11 – Sequence identity of Ag SPR3p with N. crassa CDC12p using UniProtKB: Identical matches are shown in the figure.
Characterization of plasmid BB

The plasmid BB is also a derivative of the original integrative plasmid pSV 2. In this plasmid, the RIB 3 promoter was replaced by the A. gossypii RAD 14 gene/promoter homologue, the Lac Z gene replaced the GFP gene, and the whole fragment was cloned into the Bgl II restriction site of plasmid pSV 2.

The plasmid BB was sequenced, and the sequencing data was subjected to bioinformatics searches using the KEGG database, Unit Prot, and SGD for homology. The nucleotide sequence of both the transcribed and the flanking regions of RAD 14 revealed an 849-bp open reading frame. The gene sequence showed a high degree of homology (42%) with genes of the HOG I pathway of S. cerevisiae, which is involved in the stress response. Specifically, it plays a role in combating osmotic stress in S. cerevisiae (Nadal & Posas 2022). This pathway may be implicated in combating the hyperosmotic conditions resulting from the accumulation of intracellular riboflavin in E. ashbyii, and this could lead to further scrutiny.

Gene sequence homologous to gene for stress response protein RAD 14 like protein involved in hypoxia stress response

CGGGGGGATGCTGGGGTTCATTTCTTTGGTGATTATACGATTGTTCGGGATGGTTATATAATTCGCTGTGGTAAT
ATTGTTCGTITTTATTTCCAGCTGAATCGTTAACCACGATGCACCAATAATGGAGCACAACCTCTGCAAGTGCGG
AAAAAAATATAG
GCCGGAGACTGACGGATGACAATCTGAGGTCAGTGGTGTTGGGGAATATCGGCTATCGAGAGAAACCA
ACTGCTGTTCCCCTAAAACGTAGGACGAAGAAATGAGCTCTGTAATAGCTCTCGAGATATTCGTTATTT
GCATTGACATAGTTGG
CCTACTTCAATAGGTGTTGAACCTACAAAGAAGACTATAATCAACATAGTATGTGGCAGCTACCCCCCTGCGG
GCCGTGCAATTTTTG
GGTGGGCTACCTAGCGCAGGCTCAAGACACCCCTATATGGGAGAGGAGCTATCGGACTAATTCCAGTA
CTTTATCTATTGGGG
GAAGAGCTTCTTCCGACCTAGCCACCTGGAACAAACCCGACGAGGCACTCAGCCGCCAACTGAGCCGGCAACCTA
GTAAATTCCAAACTATTG
CAACAGGCTGTCTCTGACTCTTCCCTCTCTTAACCCAGGTAGCCATCGAAGTCAAGACTGGTCTCAGTA
AAACTACGAGGAC
CCAGTGAAGGATGGCGAGCTATAGATAGGCGCTGTATATAAGTTATATGTGTGACC
CATGCAAGTGCGCTCA ATATACCACTCAGAATTTACAG

Discussion

Characterisation of plasmid D

This study has shown that the plasmid D, which harbours the SPR 3 gene homologue, is probably a regulatory gene since it exhibits a high degree of homology to the zinc finger binding protein and the regulatory SPR 3 gene of S. cerevisiae and SPR type G gene of S. ludwigi and E. cymbalaria as well as homology to the K. marxianus CDC 3p and N. crassa CDC 12 protein. Both of these are regulatory proteins with zinc clusters and zinc finger domains in their structures. Many regulatory proteins in yeast and filamentous fungi employ zinc finger motifs and zinc clusters for mediating transcriptional regulation. Zinc clusters are especially implicated in the regulation of amino acid and vitamin biosynthesis (MacPherson et al. 2006, Schillig & Morschhäuser 2013, Garcia-Estrada et al. 2018, Li & Liu 2020). Since the database search has revealed a significant degree of homology between the AgSPR 3 homologue and zinc finger proteins, it is quite possible that the Ag SPR 3 homologue encodes a transcription factor which probably regulates cell division and septation by employing the zinc finger motif/zinc cluster to mediate transcriptional regulation of these processes. This aspect of the AgSPR 3 gene can be probed further.
Characterisation of plasmid BB

This study has shown that the plasmid BB, which harbours the RAD 14 gene homologue, is probably involved in the pathway activated in response to osmotic stress. The homology searches in the databases revealed a significant degree of homology to the genes of the HOG 1 pathway of S. cerevisiae, which plays a role in combating osmotic stress (Warringer et al. 2010, Nadal & Posas 2022). Since both A. gossypii and E. ashbyii are overproducers of riboflavin, an intracellular accumulation of the metabolite could lead to a hyperosmotic condition within the hyphae, leading to osmotic stress, which could, in turn, trigger the stress response pathway. Hence, the RAD 14 gene homologue could be probed further for its role in combating osmotic stress due to intracellular accumulation of riboflavin, in addition to its possible role in combating radiation stress. The possibility of crosstalk between the osmotic stress response and radiation stress response pathways could be explored as a time course to check temporal gene regulation. Earlier reports implicate the HOG pathway’s involvement in other stress conditions, such as temperature fluctuations, oxidative stress, and heat stress, where it plays the role of a modulator in fine-tuning the response. (Winkler et al. 2002, Bilsland et al. 2004, Panadero et al. 2006, Gutin et al. 2015). The RAD 14 gene in E. ashbyii and A. gossypii can be studied for such a modulating role.

Conclusions

Knowledge about the biochemistry and genetics of riboflavin overproduction by E. ashbyii is of interest both from a fundamental and an applied point of view. A host of cellular processes are involved in the synthesis and secretion of riboflavin. Of these, the process of intracellular compartmentalization and septation is very important. In the present study, a plasmid bearing the AgSPR 3 gene (which functions in septation) fused to the Lac Z reporter gene was characterized, and it showed homology to the S. ludwigii and E. cymbalariae septin protein, S. cerevisiae SPR 3 gene, and K. marxianus CDC 3 and N. crassa CDC 12 genes involved in cell cycle regulation, a possibility that can be explored further. Riboflavin is an essential vitamin, but it causes photo-induced damage to the DNA at higher concentrations. One of the mechanisms of repairing the damaged DNA is by RAD proteins. Hence, in this study, the plasmid bearing the Ag RAD 14 gene fused to the Lac Z reporter gene was characterized, and it showed homology to the S. cerevisiae HOG 1 gene, which could prove to be the focus of future research. Finally, molecular tools for E. ashbyii have been characterized in this study and this would aid in carrying out future studies on the biochemistry and genetics of flavinogenesis by E. ashbyii. In future, these vectors will be used to transform E. ashbyii and pull out the homologous genes to study their respective roles in E. ashbyii.

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References


