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Changes in the bacterial community associated with hydra during reproduction

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Disciplines
Microbial Ecology

Keywords
Microbiota Change
Hydra
Microbiota
Budding

Type of Observation
Standalone

Type of Link
Orphan Data

Submitted Apr 13, 2017
Published Oct 26, 2017

Triple Blind Peer Review
The handling editor, the reviewers, and the authors are all blinded during the review process.

Full Open Access
Supported by the Velux Foundation, the University of Zurich, and the EPFL School of Life Sciences.

Abstract

Hydra is an evolutionarily ancient multicellular organism which has been used as a model system in developmental biology. Previously, a study has shown that microbes or microbial factors may play an important role in asexual reproduction by budding of hydra. However, no attempts were made to identify the microbes associated with hydra during budding. In the present study, we attempted to explore the microbial community structure of budding hydra using Ion Torrent PGM. Our study showed notable differences in the microbiota of budding and non-budding hydra polyps. Differences in many bacterial taxa were also observed when microbiota of sexually matured male polyps was compared with non-reproductive polyps. The present study brings out significant differences in the microbial community structure of hydra during reproductive stages. Elucidating the possible role(s) of microbes in the reproduction of hydra may provide an interesting insight into the evolution of interactions between microbes and multicellular animals.

Introduction

The microbiota associated with a host organism is known to aid many physiological processes including nutrition, immunity, resistance against pathogen, reproduction, and development [2]. In fact, host microbiota interaction is one of the significant events in evolution and occurs in organisms ranging from single-celled amoeba to humans. Many organisms have developed specialised mechanisms that assure persistence of the symbiosis in the host [3]. Hydra, an evolutionarily ancient multicellular organism, also harbours microbes which are believed to aid several vital processes such as immunity, cellular composition, proliferation and budding [4]. This association appears to be species-specific and is believed to be controlled by species-specific anti-microbial peptides called arminins [5]. Although Fraune and Bosch (2007) [6] showed that hydra polyps maintained in the laboratory for long periods of time exhibit similarities in microbial composition with their counterparts from the wild, a recent study suggested the influence of external environment on the microbial community structure of laboratory and wild populations of *Hydra vulgaris* Ind-Pune [7].

The role of microbes in the major life processes of hydra was demonstrated by Rahat and Dimentman [8] who observed a reduction in budding in hydra grown in a germ-free medium. Further, on inoculation of non-sterile medium budding resumed, suggesting a role of microbes and/or microbial factors in the event.

Objective

Although a lot has been speculated about the role of bacteria in budding in hydra, there are no reports on either the microbial compositions of budding polyps or their possible role in budding. The present study is the first step in understanding the differences in microbial composition of hydra polyps during non-reproductive stage as compared to budding or asexual reproductive stage.

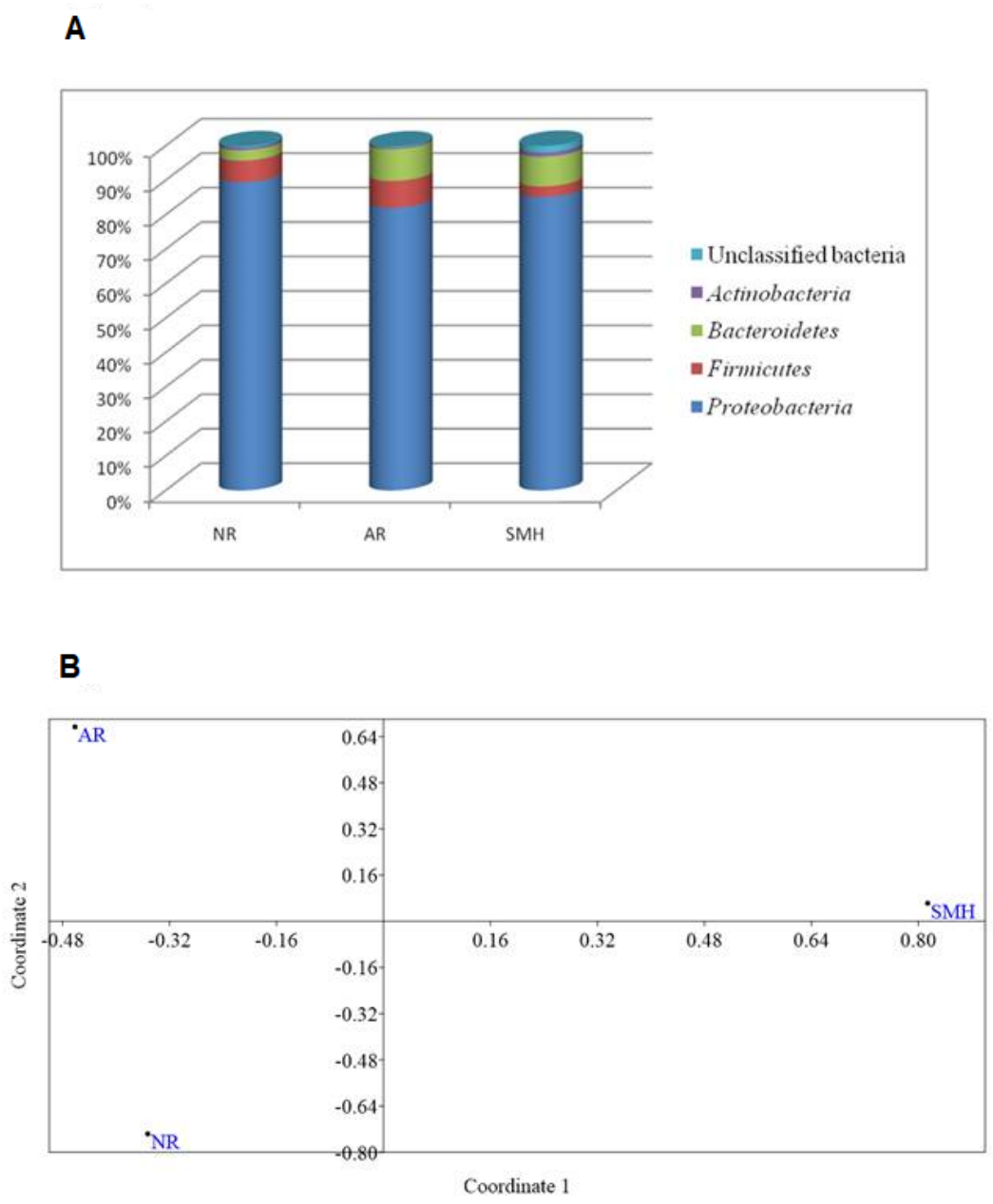

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Figure Legend

Figure 1. Differences in microbial composition of non-reproducing, budding, and male gonad-bearing hydra polyps.

(A) Taxonomic distribution of bacterial groups (at phylum level) of samples. The abundance of the phyla is plotted on the Y–axis. Unclassified bacteria are sequences without any match in the database. Proteobacteria are most dominant phylum in all samples. Colour panel on the right indicates colour code for respective phylum.
NR: Non-reproductive hydra polyps, AR: Asexually reproductive (i.e. budding) hydra polyps, SMH: sexually matured male hydra polyps.

(B) PCoA plot based on the microbial composition of the samples. Microbial community of samples separated from each other based on their reproductive

stages.
NR: Non reproductive hydra polyps, AR: Asexually reproductive (i.e. budding) hydra polyps, SMH: sexually matured male hydra polyps. PCoA plot was constructed using PAST software [1].

Results & Discussion

Samples studied by us showed a total of four bacterial phyla (Fig. 1A). Among them phylum *Proteobacteria* was highly abundant (82%–90%) followed by *Bacteroidetes* (3%–9%) and *Firmicutes* (3%–8%). These three phyla contributed more than 97% of the total bacterial community. All the samples in the present study were found to be dominated by *Proteobacteria.* Compared to NR polyps, AR polyps exhibited less abundance of *Proteobacteria*, though this remains the most abundant phylum. Such high abundance of *Proteobacteria* was also seen in the laboratory-maintained and wild populations of hydra [7]. Interestingly, Franzenburg et al. [9] found newly hatched hydra to be abundant in bacteria belonging to phylum *Bacteroidetes.* We also found an increase in the abundance of *Bacteroidetes* in budding as compared to non-budding polyps suggesting a change in the microbial composition of hydra during asexual reproduction.
Principle coordinates analysis (PCoA) plots also showed predominant differences in the microbial composition of NR and AR polyps (Fig. 1B) suggesting the possible influence of budding on the microbiota of hydra or vice versa. The shift in the composition of microbes was also reflected in an altered abundance of different bacterial groups (Table 1). Although microbes belonging to *Oxalobacteraceae* are the routinely found microbes in hydra, studies in the past have suggested that high abundance of *Oxalobacteraceae* in hydra polyps inoculated with foreign microbiota [5]. The precise role of *Oxalobacteraceae* cannot be ascertained with the available data but an increased abundance of microbes belonging to *Oxalobacteraceae* clearly suggests a change in the microbial composition of hydra during reproduction. Likewise, *Pseudomonadaceae, Sinobacteraceae, Rhodobacteraceae,* and *Cytophagaceae* also showed increased abundance in the budding polyps. Microbes belonging to *Rhizobiales*, on the other hand, showed higher abundance in the non-budding polyps.
Fraune et al. [10] found a correlation between the cellular composition of hydra and its microbial community structure. Disturbance in cellular composition of hydra leads to a change in its microbial community structure. The budding event is characterised by changes in the cellular composition of hydra, especially it is associated with a significant rise in the number of interstitial cells and its derivatives [11]. This could be the possible reason for alteration in the microbiota of hydra during budding. It has been speculated that bacteria or factors derived from bacteria may be necessary for epithelial homeostasis and proliferation leading to budding in hydra [12] [10]. In this study, we have shown for the first time that there is a significant change in the microbial composition of hydra polyps during budding. Our data demonstrate changes in several bacterial groups during budding, which could be directly or indirectly influencing the budding process itself. Moreover, Fraune et al. [10] showed that removal of interstitial cells is associated with the decrease of microbes belonging to P*roteobacteria.* Based on this study, the increase in the abundance of bacteria belonging to *Proteobacteria* during budding of hydra observed by us could be due to an increase in interstitial cells.
As is the case for budding polyps, there is no information about the microbial community structure of hydra when it attains sexual maturity. Fortunately, we came across sexually mature male hydra (SMH) in our lab. The sexual maturity can be easily confirmed visibly by occurrence of motile sperm in the testes. When the microbial composition of male polyps was studied; to our surprise, we found significant differences in its microbial composition as compared to both non-budding (non-gonad-bearing) polyps and budding polyps (Fig. 1A and B, Table 1). At the deeper taxonomic level, SMH polyps showed a high prevalence of *Oxalobacteraceae, Sinobacteraceae, Rhizobiales, Comamonadaceae,* and *Methylophilaceae.* Franzenburg et al. [9] have shown the temporal variation of microbial community structure of hydra during embryogenesis suggesting the possible influence of microbiota on the development of hydra. In fact, like many other organisms, hydra is also considered as the metaorganism wherein there is a continuous interaction between the host and its microbiota. The present observation on changing

microbiota during asexual reproduction and sexual maturity strongly indicates a role of microbes in reproduction in hydra. It may be mentioned that despite several attempts we were unable to get any sexual matured female polyps.

Conclusions

Studies in the past have shown that microbiota not only provides fitness benefit to the host but also plays a significant role in its reproduction [13]. To our knowledge, this is the first study reporting clear changes in microbial content during asexual reproduction and sexual maturity indicating microbial influence over a major life event. Our findings provide a framework for undertaking detailed studies to elucidate the role(s) of associated microbes in budding in hydra. Studies to explore possible cross-kingdom signalling as a mode of communication between the host and the associated microbes during important life events would be highly rewarding.

Limitations

In the present study, our analysis is limited to the stage at which there is the first sign of the budding. The exploration of microbial community structure at different stages of budding would shed more light on its role in budding of hydra. In the present study, we have explored the microbial community structure of reproducing males. However similar analysis in reproducing females remains to be carried out.

It has been shown that budding occurs under favourable conditions and is directly proportional to feeding [14]. However, the precise signalling molecule(s) involved in this phenomenon is yet to be identified. Studies have shown that metabolites of arachidonic acid (AA) play an important role in tentacle regeneration, cell proliferation and budding in hydra [15]. Especially, 12 HETEs (hydroxy-eicosatetraenoic acids), metabolites of lipoxygenase activity, are known to induce budding in hydra [15]. The observed increase in the abundance of order *Proteobacteria* during budding seems relevant as the members of this order are known to produce lipoxygenase enzymes [16]. Further, AA and its metabolites are also known to induce chemotaxis, cell proliferation, regulation of enzymatic activity and cAMP formation [17]. Budding in hydra is initiated by movement of cells at the point of budding, followed by differentiation of interstitial cells into the bud region. In this regard, AA and its metabolites could be key triggers for budding. Although the presence of AA and its metabolites have already been shown in hydra polyps [17], their source is not known. *Artemia* larvae used as a feed for *Hydra* are a rich source of AA [18]. It is likely that bacteria belonging to phylum *Proteobacteria* help in the breakdown of AA present in *Artemia* to produce its metabolites such as HETEs that result in the induction of budding. Suppression of budding in hydra on starvation [12] supports this conjecture. AA and its metabolites play a key role in the metamorphosis of *Hydractinia echinata* [19] and in starfish, these are known to control egg maturation [20]. These studies also point towards the role of symbiotic bacteria in the reproduction of host. However, further detailed studies are required to elucidate the precise mechanism involved.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201706000004.

Funding Statement

This study was funded by extramural grants from Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Government of India and Emeritus Scientist grant from Council for Scientific and Industrial Research (CSIR), New Delhi to SG and a DST-PURSE grant to SSG. YSS wishes to thank Department of Biotechnology (DBT), Government of India for financial support.

Acknowledgements

We would like to acknowledge Mr. Somak P. Chowdhury for his help during sequencing.

Ethics Statement

Not Applicable.

Citations

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