Himalaya, India, with receiver function CCP stacking. *Earth Planet. Sci. Lett*., 2013, **367**, 15–17.
40. Godard, V. and Burbank, D. W., Mechanical analysis of controls on strain portioning in the Himalayas of the central Nepal. *J. Geophys. Res*., 2011, **116**, B10402, doi: 10.1029/2011JB008272.
41. Lee, J. W., Hamada, M., Tabuchi, G. and Suzuki, K., Prediction of fault rupture propagation based on physical model tests in sandy soil deposit. In 13th World Conference on Earthquake Engineering Vancouver, Canada, 1–6 August 2004, paper no. 119.
42. Lemonnier, C. *et al.*, Electrical structure of the Himalaya of Central Nepal: high conductivity around the mid-crustal ramp along the MHT. *Geophys. Res. Lett*., 1999, **26**, 3261–3264.
43. Singh, D. D. and Gupta, H. K., Source dynamics of two great earthquakes of the Indian subcontinent: the Bihar–Nepal earthquake of 15 January 1934 and the Quetta earthquake of 30 May 1935. *Bull. Seismol. Soc. Am*., 1980, **703**, 757–773.
44. Wessel, P. and Smith, W. H. F., New improved version of generic mapping tools released. *Eos Trans*., 1998, **79**(47), 579; doi: 10.1029/98EO00426.

ACKNOWLEDGEMENTS. This work was carried out jointly by SAC (ISRO) and IIG (DST) as part of the DMSP R&D programme. Tapan Misra, P. K. Pal and Manab Chakroborty (SAC, Ahmedabad) have supported the collaborative studies. Figures were prepared using GMT software of Wessel and Smith[44].

Received 5 December 2015; revised accepted 17 March 2016

doi: 10.18520/cs/v111/i5/913-919

Rumen virome: an assessment of viral communities and their functions in the rumen of an Indian buffalo

Nidhi R. Parmar, Subhash J. Jakhesara, Amitbikram Mohapatra and Chaitanya G. Joshi*

Department of Animal Biotechnology,
College of Veterinary Science and Animal Husbandry,
Anand Agricultural University, Anand 380 001, India

Viruses play a key role in compensating bacterial population in any ecosystem of the planet. Rumen, a highly diverse ecosystem, is still under-explored for viral communities and their metabolic capabilities. We carried out shotgun sequencing of enriched viral particles from rumen fluid collected from an Indian buffalo. The study revealed that well-assembled contigs of Newbler and Velvet got majority of assignments to virus domain that further revealed *Caudovirales* as a major order. A majority of the Firmicutes bacteriophages were found in the study, which also confirm the presence of conserved domains such as peptidases against Firmicutes phages.

*For correspondence. (e-mail: cgjoshi@rediffmail.com)

Keywords: Bacteriophage, contigs, gene prediction, peptidase, virome.

THE rumen is a highly diverse environment encompassing bacteria, archaea, eukaryota and viruses. Several studies have examined the structure of rumen bacterial communities with insight into their efficiency of carbohydrate utilization[1,2]. Additionally, numerous new fungal communities have been reported using 18S r-RNA gene[3]. Despite their global abundance[4], the viral communities are still underexplored from any particular niche. Various microbial ecosystems such as soil associated ecosystem[5], aquatic microbial ecosystem[6] and gut microbial community[7] have been studied for viruses using conventional methods.

With the advent of next generation sequencing (NGS) technology, it has now become easier to efficiently study the viral community from any niche at greater depth even if their host bacterium is uncultivable. Although there are many reports that have deciphered the viral community in bovine rumen including phage–bacteria relationship[8,9], so far no study involving exploration of the viral community from Indian ruminants has been reported. The present study attempts to enrich bacteriophages from the rumen of an Indian buffalo, with further exploration of taxonomy of rumen virome, the probable hosts and the rumen virome metabolic profile.

The present study included a Surti breed of buffalo reared at Animal Nutrition and Research Station (ANRS), Anand Agricultural University (AAU), Anand, the diet of which mainly included forage-based diet before sample collection. All experimental procedures involving animals were conducted with prior approval by the University Animal Ethics Committee (permit number: AAU/GVC/CPCSEA-IAEC/108/2013), Anand Agricultural University (AAU), Anand, Gujarat, India.

Rumen fluid (~500 ml) was collected using flexible stomach tube after 2 h of feeding[10]. The rumen fluid was brought to laboratory under refrigerated condition after fractionation using two-layered muslin cloth to remove larger solid particles of feed. First, 100 ml of rumen fluid was centrifuged at 5000 *g* for 10 min, to remove larger particles. The supernatant was collected and filtered with 3 μm filter followed by centrifugation at 5000 *g* for 10 min. Again, the supernatant was filtered through 0.22 μm filter and transferred into two Vivaspin 20, 30 kDa molecular weight cutoff columns (~15 ml each) for centrifugation at 8000 *g* for 15 min and the particles above 30 kDa were enriched by retaining 1 ml of the concentrate from top of the column[11]. The concentrate was then given DNase I (at final concentration of 10 μg/ml) and RNase A (at final concentration of 10 μg/ml) treatment for 1 h at 37°C followed by enzyme inactivation at 65°C for 10 min.

Total DNA from the nuclease-treated rumen virome concentrate was isolated using High Pure Viral Nucleic

Table 1. Assembly of rumen virome sequences using different assemblers

	Newbler	MetaMOS	Velvet
Input number of reads	259,419	259,419	259,419
Input number of bases	69,871,078	69,871,078	69,871,078
Number of aligned reads	7446 (2.93%)	72,484 (27.9%)	180,334 (69%)
Number singletons	236,500	186,935	79,085
All contigs			
Number of contigs	938	58,697	1816 (kmer 45)
Average contig size	798	201	642
N50 contig size	770	215	614
Large contigs			
Largest contig size	4589	1763	2103
Number of contigs above 1 kb	59	28	85

Table 2. BLASTx statistics of all the contigs obtained from different assemblies

		Newbler	MetAMOS	Velvet
NR database	% of contigs assigned out of total contigs	89.13	20.94	57.87
	% of contigs matched to phages/viruses out of assigned contigs	7.18	8.42	9.61
Virus database	% of contigs assigned out of total contigs	21.96	6.65	21.42

Acid kit (Roche Applied Science, Germany)[12]. Total rumen virome DNA was quantified using Qubit 2.0 (Invitrogen) high sensitivity kit. Approximately 300 ng of total rumen virome DNA was used as an input for the shotgun DNA library preparation for an Ion Torrent PGM platform (400 bp chemistry). The sequencing procedures were carried out according to the manufacturer's instructions.

A total of 259,419 sequences were obtained and they were further filtered for base quality using PRINSEQ[13] for an average Phred quality score of minimum 20 (ref. 9). The filtered reads were assembled to generate the contigs using multi-assembler approach using Newbler assembler (version 2.6) (ref. 14), Velvet assembler V1.2.06 (ref. 15) and MetAMOS (ref. 16). Velvet modules were run with different k-mer lengths (21, 27, 33, 39 bp) with the following parameters: Velveth K = 21–39, -short and velvetg-exp_cov auto, -cov_cutoff 0 -scaffolding no. This was followed by pooling of all the assemblies obtained from different k-mer sizes, with larger k-mer size of 45. The default parameters were used for assembling the reads in Newbler (minimum overlap length = 40, minimum overlap identity = 90, seed length = 16) and MetAMOS (initPipeline-q-1-a SOAPdenovo2).

The assembly done by various assemblers was annotated using BLASTx against non-redundant (nr) Reference Sequence (RefSeq) database and customized virus database (generated by downloading the virus genomes from NCBI) using e value <0.00001. To improve the annotation, gene prediction was performed using FragGeneScan[17] for all various assemblies. The contigs were annotated using BLASTx against nr database (e value <0.00001) and the output was imported into MEGAN version 5.2.3 (ref. 18) to visualize the taxonomical and functional assignment. Moreover, the probable bacterial hosts of the rumen viruses were identified by performing BLASTn against nr database (e value <0.00001). The best assemblies were then translated into six open reading frames, which further assigned for the conserved domain annotations using CD-search NCBI (e value = 0.00001)[19]. The raw sequence data of rumen virome sample used in this study is available on the EBI metagenomics (European Bioinformatics Institute) server, (Sample ID: ERS712566, Run ID: ERR864205).

The assembly resulted in the generation of 938 (2.93% reads) contigs in Newbler assembler, 1816 (69% reads) contigs in Velvet assembler and 58,697 (27.9% reads) contigs in MetAMOS (Table 1). The average contig size was found to be highest in Newbler (798 bp) compared to Velvet (642 bp) and MetAMOS (201 bp). Further details on assembly statistics is as shown in Table 1.

BLASTx was carried out using two different databases (nr database and customized virus database). BLASTx against nr database revealed that out of total number of contigs, 89.13% of contigs obtained from Newbler, 57.87% from Velvet and only 20.94% from MetAMOS were assigned to the nr database annotations (Table 2). However, of the total assigned contigs to the nr database, 7.18%, 8.42% and 9.61% from Newbler, MetAMOS and Velvet respectively, showed hits to viruses and phages. On the other hand, BLASTx output with virus database revealed that contigs from Newbler (21.96%) and Velvet (21.42%) were assigned more compared to MetAMOS (6.65%) (Table 2).

The contigs were predicted for coding sequences to check whether the BLASTx results obtained with contigs

are being improved with gene predictions. The analysis revealed that the annotations to virus database increased for the contigs generated from Newbler (72.85%) and Velvet (69.57%) whereas the opposite was observed for the contigs generated from MetAMOS assembly (Figure 1). Further analysis was performed by taking the predicted contigs into consideration.

The taxonomical assignments of the gene predictions revealed majority of the annotations to the virus kingdom (Table 3). The analysis assigned majority of annotations to double stranded DNA viruses where the abundance percentage of the contigs assigned were 51.95%, 48.59% and 4.98% for Newbler, Velvet and MetAMOS respectively (Table 4). Sub classification of double stranded DNA viruses to order level revealed *Caudovirales* as the most abundant order in the rumen with 47.21% abundance in the contigs obtained from Newbler, 83.85% abundance in the contigs obtained from MetAMOS and 56.27% abundance in the contigs obtained from Velvet (Figure 2). Further the order Caudovirales was subclassified to family level where two families were observed with major assignments, i.e. Myoviridae (Newbler = 31.73%, MetAMOS = 26.61% and Velvet = 34.41%)

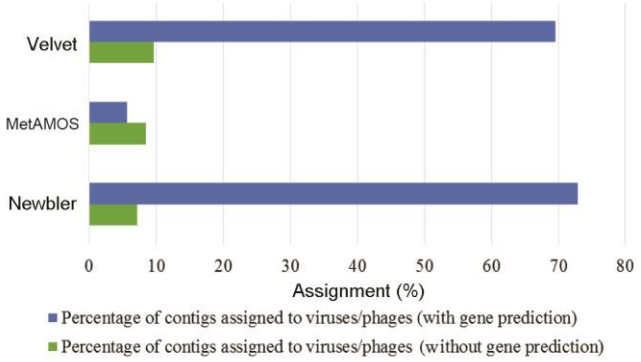

Figure 1. Comparison of assignment percentage of the contigs (without gene prediction and with gene prediction) to the viruses/phages.

Table 3. BLASTx statistics of all contigs (with gene prediction) obtained from different assemblies

	Newbler	MetAMOS	Velvet
Genes predicted	1105	57,643	2264
Assigned to viruses	805	3239	1575
Assignment to viruses (%)	72.85	5.62	69.57

Table 4. Taxonomical assignment of the predicted contigs to viruses

	Newbler (% abundance)	MetAMOS (% abundance)	Velvet (% abundance)
dsDNA virus	51.95	4.98	48.59
ssDNA virus	0.72	0.01	0.31
ssRNA virus	4.71	0.00	3.31

and Siphoviridae (Newbler = 42.44%, MetAMOS = 58.28% and Velvet = 42.16%) (Figure 3).

The contigs were also assigned to SEED database for examining the metabolic potential of viral genes. The analysis of virome metabolic profile showed that, the contigs were assigned to nucleoside and nucleotide metabolism (Newbler = 0.09%, MetAMOS = 0.00% and Velvet = 0.09%), protein metabolism (Newbler = 0.00%, MetAMOS = 0.01% and Velvet = 0.13%), regulation and cell signalling (Newbler = 0.09%, MetAMOS = 0.02% and Velvet = 0.18%), phages, prophages and transposable elements (Newbler = 2.81%, MetAMOS = 0.15% and Velvet = 2.65%), phages, prophages, transposable elements and plasmids (Newbler = 0.90%, MetAMOS = 0.07% and Velvet = 0.93%), membrane transport (Newbler = 0.63%, MetAMOS = 0.03% and Velvet = 0.71%), DNA metabolism (Newbler = 0.90%, MetAMOS = 0.08% and Velvet = 1.10%) and virulence, disease and defence (Newbler = 0.00%, MetAMOS = 0.01% and Velvet = 0.13%) (Table 5).

In order to decipher probable bacterial hosts, BLASTn of the predicted contigs was performed. The results showed that the bacteriophages acting on Firmicutes phylum were more abundant in all three assemblies with the highest annotations in Velvet assembly (40.03%) than in MetAMOS (38.43%) and Newbler (33.01%) (Figure 4). However, we also observed the bacteriophages acts on Proteobacteria phylum at subdominant level with 28.71% annotation in Newbler assembly, 24.06% in MetAMOS assembly and 21.51% in Velvet assembly (Figure 4).

The contigs obtained from Newbler and Velvet assembler were subjected to conserved domain search where several putative domains belonged to viruses were found. Such putative domains for Newbler assembled contigs included terminases, peptidase U35, phage holin 3, DNA breaking and re-joining enzymes (topoisomerase and integrase), bacterial Ig-like domains, virion protein, DNA/RNA non-specific endonucleases, DNA encapsidation protein from podoviruses (Gp16), peptidoglycan-binding domains, site-specific serine recombinases, phage head and tail joining protein, phage major capsid protein E, peptidase M23, phage head and tail connector protein (Gp10) and resolvase family (Figure S1, see Supplementary Material online).

The contigs obtained from velvet showed major putative domains like DNA breaking and re-joining enzymes (topoisomerase and integrase), baseplate J-like protein, caudovirus prohead protease (peptidase U35), CRISPR/Cas system-associated protein Cas4, TopoIIA Trans DNA gyrase (predominant form of topoisomerase and are found in some bacteriophages, viruses and archaea, and in all bacteria and eukaryotes), reverse transcriptase like superfamily, HIRAN domains (in bacteria and prophages), recombinases (integrases/recombinases of mobile genetic elements of diverse bacteria and phages), head- tail connector protein gp6 of bacteriophage HK97, phage

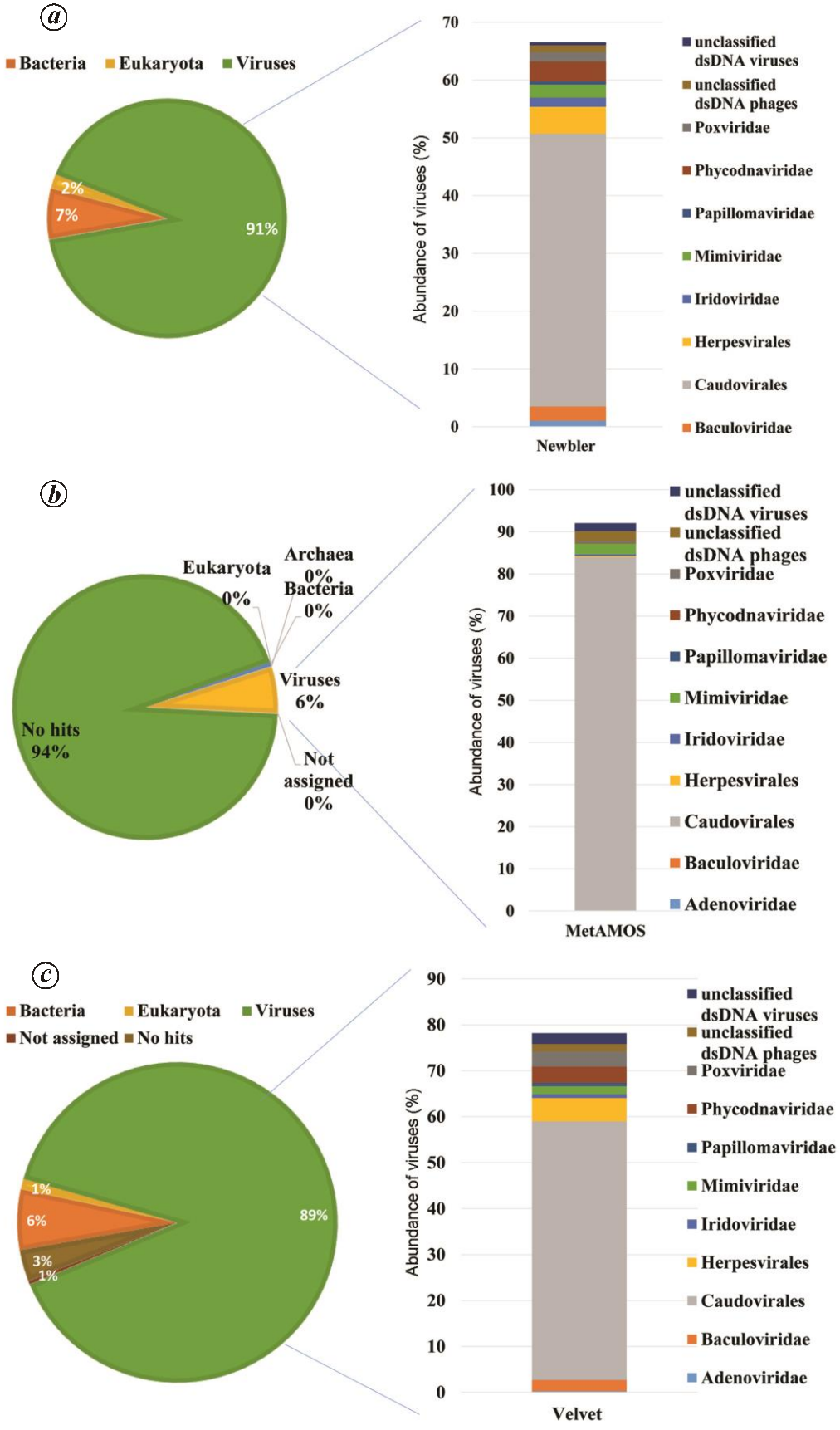

Figure 2. Taxonomical assignment of the rumen microbiome and specifically virome at family level for the reads assembled by (***a***) Newbler assembler, (***b***) MetAMOS assembler and (***c***) Velvet assembler.

Table 5. SEED subsystem based classification of different contigs

	Newbler (%)	MetAMOS (%)	Velvet (%)
Nucleosides and nucleotides	0.09	0.00	0.09
Protein metabolism	0.00	0.01	0.13
Regulation and cell signalling	0.09	0.02	0.18
Phages, prophages, transposable elements	2.81	0.15	2.65
Phages, prophages, transposable elements, plasmids	0.90	0.07	0.93
Membrane transport	0.63	0.03	0.71
DNA metabolism	0.90	0.08	1.10
Virulence, disease and defence	0.00	0.01	0.13
Not assigned	92.49	6.80	92.23
No hits	2.62	92.85	2.47

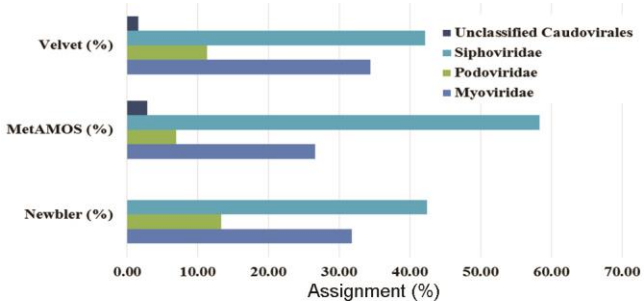

Figure 3. BLASTn output of different assemblies. X-axis: Bacteriophages acting on various genera; Y-axis: Abundance percentage of the bacteriophages.

minor capsid protein2, Cpl-7 lysozyme C-terminal domain, D5 N terminal like domain (found in D5 proteins of DNA viruses and bacteriophage P4 DNA primases phages), transglutaminase-like superfamily, phage tail tube protein, minor capsid protein, phage capsid family, phage major tail protein 2, phage integrase SAM-like domain, phage capsid family, phage integrase (N-terminal SAM-like domain), VirE N-terminal domain and serine recombinase family (catalytic domain) (Figure S2, see Supplementary Material online).

Moreover, few of the conserved domains such as recombinases, peptidases and lysozymes were checked for their maximum similarity by doing multiple sequence alignment and constructing phylogenetic tree. Out of two peptidases found in our dataset, peptidase U35 was grouped with the hypothetical protein acting on Firmicutes (Figure S3, see Supplementary Material online) and another peptidase M23 was grouped with the protein acting on CFB-group of bacteria (commonly known as Bacteroidetes) (Figure S4, see Supplementary Material online). Apart from that, putative C-terminal domain of lysozyme showed more similarity with *N*-acetylmuramidase/lysin acting on *Carnobacterium maltaromaticum*, a species of Firmicutes group (Figure S5, see Supplementary Material online). Serine recombinase showed its sequence similarity with a hypothetical protein acting on *Parimonas micra*, species of Firmicutes group (Figure S6, see Supplementary Material online).

Viral communities are the most abundant and highly diverse biological entity on the earth. Rumen is a highly diversified microbial ecosystem consisting of bacteria (10^{10}–10^{11} cells/ml, representing more than 50 genera), ciliate protozoa (10^4–10^6/ml, from 25 genera), anaerobic fungi (10^3–10^5 zoospores/ml, representing five genera) and bacteriophages (10^8–10^9/ml)[20]. Viruses play an important role in maintaining the microbial population in a rumen ecosystem, also drive the evolution through natural selection of phage-resistant microbes[21]. The NGS technologies used for exploring the microbial community from any particular niche, have made it possible to get a deeper insight into viral community and their metabolic functions.

In the present study we assembled the sequences using three different types of assemblers to check whether there is any bias in the annotation due to assembly and also to evaluate the annotation of rumen virome data. Of all three assemblers, a majority of the reads were assembled in Velvet compared to Newbler and MetAMOS. The assembly in Velvet was carried out with the increment in k-mer size and then pooled with higher k-mer size. This approach resulted in more number of contigs (1816) of 642 bp average length compared to Newbler (938) with an average contig size of 798 bp respectively. On the contrary, MetAMOS gave more number of contigs (58,697) than Newbler and Velvet but with shorter contig length of 201 bp. Thus, from these assembly statistics we were only able to state the better assembler based on its efficiency of utilizing sequences for assembly and generating larger contigs. However, on the basis of annotations only we could validate the best assembly and also check the biasness in the annotation for different types of assemblies. Hence, we performed BLASTx for all the three assemblies against nr database where, we observed major assignment for contigs generated from Newbler compared to MetAMOS and Velvet. But, if we look at the assignment of contigs to viruses or phages, more assignment was observed in Velvet assembly compared to Newbler and MetAMOS. We also performed BLASTx against custom virus database and found more assignment of Newbler and Velvet contigs compared to MetAMOS. It has

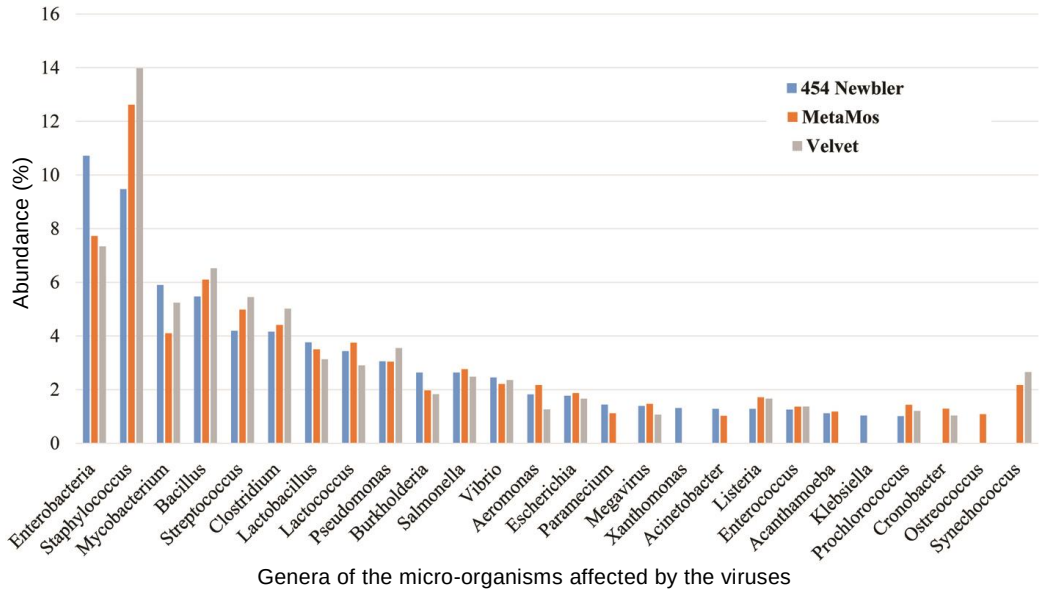

Figure 4. Percentage abundance of prophages after assigning the contigs generated from three assemblers to BLASTn.

been reported that the short contigs lead to the prediction of short and fragmented genes and thus do not exhibit any matches with reference database. Thus, the poor annotation of MetAMOS contigs may be the result of their short length[22].

Gene prediction followed by annotation, not only maximized the annotation to viruses and phages but also enabled identification of full length conserved domains of bacteriophages. A study has also explained the efficiency of gene prediction algorithm in metagenomics studies[22].

A majority of the annotations were assigned to double-stranded DNA viruses followed by single-stranded RNA viruses and single-stranded DNA viruses. A previous study on bovine rumen also showed predominance of double-stranded DNA virus[23]. By sub-classifying the double-stranded viruses at order level, we found majority of the viral hits corresponded to tailed bacteriophage[24] belonging to Siphoviridae, Myoviridae and Podoviridae families. Similar observations have been made in bovine rumen[23,25]. Moreover, the type of infected host bacteria was identified by performing BLASTn, which showed more enrichment of the *Staphylococcus* and *Enterobacter* infecting phages (Figure 4). These phages infected mainly to the genera of the phylum Firmicutes and Proteobacteria. Previous studies on Indian buffalo rumen microbiome have reported the predominance of Firmicutes, Bacteroidetes and Proteobacteria phyla and thus the concurrence of the phages with these hosts was expected[10,26]. Previous rumen virome studies also corroborate our findings where the phages infecting Bacteroidetes and Firmicutes were found to be predominant in rumen samples and thereby help in maintaining the Firmicutes to Bacteroidetes ratio in the rumen[9]. Further, to examine the phage and bacteria interaction, creation of database using virome data and annotating the contigs of microbial metagenome to that database would be helpful. A similar study has been done in the virome study by Berg *et al.*[8].

The assignment of the predicted contigs for functional annotation revealed only small percentage (6%) of significant hits to SEED database. This finding is concomitant with the previous reports on SEED assignment of the rumen virome[8]. A majority of the assigned contigs were categorized into phages, prophages and transposable elements category. This finding with large number of unannotated predicted contigs provide further evidence of more enrichment of the sequences belonging to the rumen bacteriophage. Other subsystems found in our study such as nucleotide/nucleoside metabolism, DNA metabolism and membrane transport are consistent with the virome metabolic profile described for rumen environments[8]. Complete lack of assignment of the contigs to the carbohydrate metabolism category is supporting previous reports on rumen virome studies[9] and also justifies the complete opposite metabolism profile of the rumen virome with that of the rumen microbiome where we generally find the majority of the assignment to the carbohydrate metabolism.

Further search of gene predictions was done to find putative conserved domains which showed the domains involved in the structural composition of phages (mainly tail, head, baseplate, etc.) as well as the peptidases and lysozymes acting on the bacterial cell wall. We identified two peptidases, peptidase U35 and peptidase M23, the sequences of which showed more sequence similarity with the peptidases acting on Firmicutes and Bacteroidetes respectively (Figures S3 and S4, see Supplementary Material online). Moreover, the identified recombinases and lysozymes also showed sequence similarity with the sequences of proteins acting on Firmicutes.

In conclusion, the present study showed that our data was assembled and annotated well using Newbler and Velvet. Though the reads used to generate the contigs were utilized less in the case of Newbler, the annotation percentage of those contigs to virus domain was comparable with that of Velvet assembler. Moreover, it also highlights the improvement in virome data annotation after performing the gene prediction. The rumen virome data revealed that the *Caudovirales* is the major order contributing to the overall bacteriophage population in the rumen with the Siphoviridae and Myoviridae forming the major rumen bacteriophage families. The virome data assignment also revealed Firmicutes as the major probable bacteriophage host. Moreover, the present study provides insights into bacteriophage metabolic activities where putative conserved domains such as peptidases, recombinases, lysozymes, etc. were identified. The data sequence similarity of peptidase U35 and M23 to the peptidases acting on Firmicutes and Bacteroidetes confirms the pivotal role of bacteriophages in maintaining the Bacteroidetes and Firmicutes ratio in the rumen of an animal. Thus, the present study provides insight into rumen virome community of an Indian buffalo as well as gives implications that further work on this approach can aid in identifying the bacteriophage and their putative proteins acting on methanogens to generate 'bacteriophage therapy' for reducing methanogenic activity from Indian livestock.

Competing interests: The authors declare that they have no competing interests.

Authors contributions: C.G.J. conceived this study and obtained financial support. C.G.J. and N.R.P. designed the experiment. N.R.P. carried out sample collection and wet-lab experiments. N.R.P. and A.B.M. performed bioinformatics analysis. N.R.P. wrote the manuscript. All authors have contributed and approved the final manuscript. S.J.J. helped in critical reviewing of the manuscript.

1. Hess, M. *et al.*, Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. *Science*, 2011, **331**, 463–467.
2. Brulc, J. M. *et al.*, Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. *Proc. Natl. Acad. Sci.*, 2009, **106**, 1948–1953.
3. Fouts, D. E. *et al.*, Next generation sequencing to define prokaryotic and fungal diversity in the bovine rumen. *PLoS ONE*, 2012, **7**(11), e48289.
4. Breitbart, M. and Rohwer, F., Here a virus, there a virus, everywhere the same virus? *Trends Microbiol.*, 2005, **13**, 278–284.
5. Barnet, Y. M., Bacteriophages of *Rhizobium trifolii* i. Morphology and host range. *J. Gen. Virol.*, 1972, **15**, 1–15.
6. Torrella, F. and Morita, R. Y., Evidence by electron micrographs for a high incidence of bacteriophage particles in the waters of yaquina bay, oregon: Ecological and taxonomical implications. *Appl. Environ. Microbiol.*, 1979, **37**, 774–778.
7. Flewett, T., Bryden, A. and Davies, H., Diagnostic electron microscopy of faeces i the viral flora of the faeces as seen by electron microscopy. *J. Clin. Pathol.*, 1974, **27**, 603–608.
8. Berg Miller, M. E. *et al.*, Phage–bacteria relationships and crispr elements revealed by a metagenomic survey of the rumen microbiome. *Environ. Microbiol.*, 2012, **14**, 207–227.
9. Ross, E. M., Petrovski, S., Moate, P. J. and Hayes, B. J., Metagenomics of rumen bacteriophage from thirteen lactating dairy cattle. *BMC Microbiol.*, 2013, **13**, 242.
10. Parmar, N. R. *et al.*, Metagenome of mehsani buffalo rumen microbiota: An assessment of variation in feed-dependent phylogenetic and functional classification. *J. Mol. Microbiol. Biotechnol.*, 2014, **24**, 249–261.
11. Hall, R. J. *et al.*, Evaluation of rapid and simple techniques for the enrichment of viruses prior to metagenomic virus discovery. *J. Virol. Meth.*, 2014, **195**, 194–204.
12. Niesters, H. G., Van Esser, J., Fries, E., Wolthers, K. C., Cornelissen, J. and Osterhaus, A. D., Development of a real-time quantitative assay for detection of epstein-barr virus. *J. Clin. Microbiol.*, 2000, **38**, 712–715.
13. Schmieder, R. and Edwards, R., Quality control and preprocessing of metagenomic datasets. *Bioinformatics*, 2011, **27**, 863–864.
14. Minot, S. *et al.*, The human gut virome: Inter-individual variation and dynamic response to diet. *Genome Res.*, 2011, **21**, 1616–1625.
15. Aw, T. G., Howe, A. and Rose, J. B., Metagenomic approaches for direct and cell culture evaluation of the virological quality of wastewater. *J. Virol. Meth.*, 2014, **210**, 15–21.
16. Treangen, T. J. *et al.*, Metamos: a modular and open source metagenomic assembly and analysis pipeline. *Genome Biol.*, 2013, **14**, R2.
17. Rho, M., Tang, H. and Ye, Y., Fraggenescan: Predicting genes in short and error-prone reads. *Nucl. Acids Res.*, 2010, gkq747.
18. Huson, D. H., Auch, A. F., Qi, J. and Schuster, S. C., Megan analysis of metagenomic data. *Genome Res.*, 2007, **17**, 377–386.
19. Marchler-Bauer, A. and Bryant, S. H., Cd-search: Protein domain annotations on the fly. *Nucl. Acids Res.*, 2004, **32**, W327–W331.
20. Hobson, P. N. and Stewart, C. S. (eds), *The Rumen Microbial Ecosystem*, Springer Science & Business Media, 1997.
21. Swain, R., Klieve, A. and Nolan, J. (eds), Factors affecting the rumen bacteriophage population. In *Australian Society of Animal Production Biennial Conference*, Australian Society of Animal Production, 1996, pp. 409–409.
22. Prakash, T. and Taylor, T. D., Functional assignment of metagenomic data: challenges and applications. *Brief. Bioinf.*, 2012, **13**, 711–727.
23. Fernando, B. R., *Metagenomic Analysis of Microbial Communities in the Bovine Rumen*, Oklahoma State University, 2012.
24. Ackermann, H.-W., Tailed bacteriophages: The order caudovirales. *Adv. Virus Res.*, 1999, **51**, P135–P202.
25. Klieve, A. V. and Bauchop, T., Morphological diversity of ruminal bacteriophages from sheep and cattle. *Appl. Environ. Microbiol.*, 1988, **54**, 1637–1641.
26. Singh, K. M. *et al.*, Metagenomic analysis of surti buffalo (bubalus bubalis) rumen: A preliminary study. *Mol. Biol. Rep.*, 2012, **39**, 4841–4848.

ACKNOWLEDGEMENTS. We thank the supporting staff at Department of Animal Nutrition, Anand Agricultural University, Anand for their support in sample collection. This work was supported by Niche Area of Excellence project funded by Indian Council of Agricultural Research, New Delhi.

Received 28 December 2015; revised accepted 5 May 2016

doi: 10.18520/cs/v111/i5/919-925