Existence of theoretical ribosomal protein mass fingerprints in bacteria, archaea and eukaryotes

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Abstract

Ribosomes are highly conserved given the importance of protein synthesis to cell survival. Although small differences in structure and functions exists in ribosomes from different species of bacteria, archaea and eukaryotes, the general structure and function remains conserved across species in the same domain of life. Thus, are ribosomal proteins that constitute ribosomes highly conserved between species in the same domain or do they possess sufficient sequence variation that help identify individual species? Having differentiated sequence would mean that ribosomal proteins from different species might account for differences in structure and function of the ribosomes in different species. Using ribosomal protein amino acid sequence information from Ribosomal Protein Gene Database for calculating molecular mass of ribosomal proteins, this study sought to determine if the molecular mass of a set of ribosomal proteins from a species could constitute a unique ribosomal protein mass fingerprint. In addition, the question of whether unique ribosomal protein mass fingerprint exists between different species in the three domains of life was also examined. Results revealed that distinct molecular mass of individual ribosomal protein could aggregate into a unique ribosomal protein mass fingerprint for individual bacterial, archaeal and eukaryotic species. Such ribosomal protein mass fingerprints could potentially find use in microbial identification through gel-free matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) profiling of solubilized ribosomal proteins. Obtained ribosomal protein mass spectrum could be compared with those catalogued in a reference database of known microorganisms where pattern recognition algorithms could determine a match. Additionally, existence of theoretical ribosomal protein mass fingerprint across species in the three domains of life also pointed to the presence of small differences in structure and function of both the large and small ribosome subunit. Such differences could reveal possible differentiated ribosomal structure and function in different species even though the general structure and function of the ribosome is conserved across species. Collectively, distinct molecular mass of individual ribosomal proteins in species pointed to a unique ribosomal protein mass fingerprint that could find use in microbial identification through gel-free mass spectrometry analysis of solubilized ribosomal proteins. Differences in mass of ribosomal proteins across species also highlighted existence of ribosomes of differentiated structure and function between different species even though the general structure and function of the ribosome remains highly conserved.

Keywords: ribosome, large subunit, small subunit, ribosomal protein, mass spectrometry, MALDI-TOF MS, microbial identification, pattern recognition, ribosomal protein mass fingerprint, ribosome typing,

Subject areas: microbiology, biochemistry, bioinformatics, biotechnology, cell biology,

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Highlights

1) Distinct molecular mass was observed for individual ribosomal protein in the large and small ribosome subunit of bacterial, archaeal and eukaryotic species.

2) Thus, a unique ribosomal protein mass fingerprint exists for the set of ribosomal protein in the large and small ribosome subunit of individual species.

3) Existence of ribosomal protein mass fingerprint in different species from the three domains of life offered a potential method for microbial identification through gel-free MALDI-TOF MS profiling of solubilized ribosomal proteins.

4) In addition, differences in molecular mass of the same ribosomal protein of different species highlighted differentiated amino acid sequences that could possibly translate into structural and functional differences in ribosomes of different species.

5) Aggregating across the set of ribosomal proteins that constitute a ribosome, differences in amino acid sequence and molecular mass of ribosomal proteins pointed to existence of species-specific small differences in ribosome structure and function even though the general role and structure of the ribosome remained highly conserved.

Significance of the work

Important cellular processes such as protein synthesis naturally demand high conservation of the constituent proteins and molecular machines that partake in the process. Thus, ribosomes and their constituent ribosomal proteins should be highly conserved from the evolutionary perspective. Specifically, the shape and function of ribosomes which are dependent on the ribosomal proteins should be similar across species in the same domain of life. However, calculation of molecular mass of ribosomal proteins in the large and small ribosome subunit of species across the three domains of life revealed distinct molecular mass of ribosomal proteins that constitute unique theoretical ribosomal protein mass fingerprint of different species. This suggested that the ribosomal proteins of different species encode a more varied amino acid sequence and richer evolutionary history than previously thought, which holds important implications for the structure and function of ribosomes. Known to be highly conserved, differentiation of the conserved general structure and function of the ribosome could exist due to the presence of myriad ribosomal protein of varied amino acid sequence. Thus, diversity in structure and differentiated function of the ribosome could exist in individual species. Presence of unique theoretical ribosomal protein mass fingerprint also point to the possibility of microbial identification, where gel-free mass spectrometry workflow utilizing matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) could profile solubilized ribosomal proteins that collectively bear a phylogenetic stamp of the species.

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Introduction

Highly conserved proteins lack sequence variation that helps chronicle evolutionary trajectory traversed by the protein. Similarly, essential cellular functions such as protein synthesis are also performed by molecular machineries finely tuned for the task through evolution. Specifically, macromolecular complexes such as ribosomes that perform essential cellular functions are unlikely to be highly divergent in structure and function given the importance of biological structure in lending functionality to the complex. Thus, given the importance of ribosomes to protein synthesis, its structure should be highly conserved. But, could the same be said of the ribosomal proteins that constitute the ribosome even though ribosomes from the bacteria, archaea and eukaryotic lineage are highly conserved in structure and function?

The answer could be gleaned from molecular phylogenomics studies that aimed to understand the evolutionary significance of ribosomal proteins that constitute the ribosome.[1] Results indicated that ribosomal proteins are endowed with sufficient sequence variation that help chronicle the evolutionary processes and natural selection pressure that act on the proteins.[2] Thus, while conserved in sequence to a large extent, ribosomal proteins are sufficiently varied that helped provide phylogenetic information of individual microbial species. Specifically, ribosomal proteins could be used collectively as markers for the evolutionary divergence between different species.[3] Hence, ribosomal proteins are at the same time conserved and yet possess sufficient sequence variation to help encode the effects of evolutionary forces on the developmental trajectory of the protein, which enables ribosomal proteins to be used as phylogenetic markers for different microbial species.[4]

Given that differences in protein amino acid sequence is likely to result in ribosomal proteins of different species to be of different molecular mass, taking the collective set of ribosomal proteins of the large and small ribosome subunits would thus provide a unique ribosomal protein mass fingerprint for individual species. Specifically, modern mass spectrometry tools such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is able to profile ribosomal proteins at mass resolution of at least 1 Da, which provides a means for determining the ribosomal protein mass fingerprint of the large and small ribosome subunit of individual species after solubilization of the proteins from the fractionated ribosome and direct MALDI-TOF MS analysis.[5 6 7] Since MALDI-TOF MS is a gentle ionization technique that does not fragment the molecular ion of the protein, data interpretation of the mass spectra obtained would be relatively easier compared to those obtained by other mass spectrometry methods such as electrospray ionization mass spectrometry (ESI-MS) which introduces multiple charging and protein fragmentation during the ionization process.

Thus, the objective of this study is to assess the feasibility of profiling a species-specific ribosomal protein mass fingerprint of the large and small ribosome subunit of a species via

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MALDI-TOF MS analysis of solubilized ribosomal proteins. To this end, the theoretical ribosomal protein mass fingerprint of the large and small ribosome subunit of different species of bacteria, archaea and eukaryotes were calculated based on amino acid sequence information of the ribosomal proteins available in the Ribosomal Protein Gene Database.[8] Obtained results revealed that ribosomal protein molecular mass differed between the same protein of different species of bacteria, archaea and eukaryotes. More importantly, unique ribosomal protein mass fingerprint of the large and small ribosome subunit exists for individual species of bacteria, archaea and eukaryotes; thereby, raising the possibility of its use as a marker for microbial identification after MALDI-TOF MS analysis of solubilized ribosome proteins. At another level, existence of unique ribosomal protein mass fingerprint of the large and small ribosome subunit of individual species of microbes also suggests possible differences in the structure and functions of ribosome of different species. Thus, although the general structure and function of the ribosome is highly conserved across species, small differences in structure and function could still be present given the presence of unique sets of ribosomal proteins.

Materials and Methods

Amino acid sequence of ribosomal proteins of different species was obtained from the Ribosomal Protein Gene Database (http://ribosome.med.miyazaki-u.ac.jp/). Molecular mass of the ribosomal proteins was calculated using the Compute pI/Mw tool at (https://web.expasy.org/compute_pi/). Species profiled include: *Escherichia coli* K-12, *Bacillus subtilis*, *Thermus thermophilus* HB8, *Synechocystis* sp. PCC 6803, *Sulfolobus tokodaii*, *Pyrococcus horikoshii*, *Methanococcus jannaschii*, *Halobacterium salinarum* NRC-1, *Neurospora crassa*, *Fusarium graminearum*, *Cryptococcus neoformans*, and *Yarrowia lipolytica*.

Results and Discussion

Table 1: Comparison of molecular mass (Da) of ribosomal proteins in the small ribosome subunit of bacterial species

Protein	*Escherichia coli* K-12	*Bacillus subtilis*	*Thermus thermophilus* HB8	*Synechocystis* sp. PCC 6803
S1P	61158.07	42402.03	59970.52	36570.06
S2P	26743.64	27967.26	29276.69	30150.12
S3P	25983.24	24300.8	26700.99	27147.11
S4P	23469.09	22835.22	24324.31	23182.79
S5P	17603.38	17622.61	17557.41	18241.1
S6P	15187.03	11124.56	11972.77	13205.21
S7P	20019.09	17892.85	18015.9	17384.15
S8P	14126.55	14843.35	15837.51	14666.19
S9P	14856.2	14290.43	14382.58	15086.34
S10P	11735.59	11665.63	11929.92	12037.05

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S11P	13844.93	13924.95	13712.83	13761.84
S12P	13737.06	15323.84	14850.58	14176.62
S13P	13067.32	13801.03	14272.7	14539.66
S14P	11580.48	10323.2	7139.65	11853.65
S15P	10268.76	10573.16	10554.35	10373.06
S16P	9190.56	10134.81	10386.94	9556.17
S17P	9704.44	10198.93	12297.59	9288.83
S18P	8986.43	9201.85	10213.19	8380.93
S19P	10430.29	10583.25	10581.4	10290.02
S20P	9666.35	9500.02	11703.02	10670.53
S21P	8499.96	6829.98		7341.45

Table 1 shows the calculated molecular mass of ribosomal proteins of the small ribosome subunit of bacterial species. Four bacterial species were profiled for this analysis: *Bacillus subtilis*, *Escherichia coli*, *Thermus thermophilus* HB8, and *Synechocystis* sp. PCC 6803. Results revealed that the ribosomal proteins' molecular mass of each species were distinct and unique compared to those of the same protein from another bacterial species. In addition, a unique mass fingerprint exists for the ribosomal proteins of the small ribosome subunit of bacterial species.

Table 2: Comparison of molecular mass (Da) of ribosomal proteins in the large ribosome subunit of bacterial species

Protein	*Escherichia coli* K-12	*Bacillus subtilis*	*Thermus thermophilus* HB8	*Synechocystis* sp. PCC 6803
L1P	24729.64	24922.77	24830.67	25851.79
L2P	29860.44	30331.96	30468.39	30433.15
L3P	22243.52	22683.28	22376.07	22709.07
L4P	22086.53	22390.93	23202.83	23355.83
L5P	20301.57	20147.54	21029.58	22475.99
L6P	18903.78	19509.34	19531.87	19666.72
L9P	15769.06	16351.94	16365.13	18942.17
L10P	17711.59	18028.76	18533.64	18675.59
L11P	14875.38	14885.36	15505.13	14977.5
L7/L12P	12295.2	12750.66	13067.24	13259.31
L13P	16018.54	16291.96	15862.77	16990.69
L14P	13541.02	13154.27	13302.61	13262.48
L15P	14980.42	15382.65	16281.02	15194.62
L16P	15281.2	16189.98	15962.84	16034.75
L17P	14364.59	13750.76	13715.04	13228.44
L18P	12769.63	13017.86	12611.78	13204
L19P	13133.24	13729.22	17151.74	13786.24

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L20P	13496.96	13638.1	13743.17	13553.12
L21P	11564.35	11275.07	11047.13	13668.82
L22P	12226.29	12459.58	12779.99	13501.76
L23P	11199.12	10928.65	10704.72	11493.6
L24P	11316.21	11142.06	12056.52	12823
L25P	10693.44	22055.51	23204.57	
L27P	9124.47	10371.82	9508.02	9448.66
L28P	9006.49	6809.09	10978.09	8993.49
L29P	7273.45	7713	8650.21	8545.65
L30P	6541.79	6637.77	6785.11	
L31P	7871.06	7443.54	8253.45	9303.67
L32P	6446.38	6728.99	6704.98	6456.31
L33P	6371.59	5496.37	6615.83	7548.79
L34P	5380.39	5253.25	6109.34	5261.14
L35P	7288.93	7557.03	7484.1	7891.4
L36P	4364.33	4305.36	4421.34	4455.48

Table 2 shows the calculated molecular mass of ribosomal proteins in the large ribosome subunit of different bacterial species. Similar to the case for ribosomal protein in the small ribosome subunit of *E. coli* K-12, *B. subtilis*, *T. thermophilus* HB8, and *Synechocystis* sp. PCC 6803, unique mass was found for individual ribosomal proteins in the large ribosome subunit of each species that differed from that of the same protein in another species. This highlighted the existence of unique ribosomal protein mass fingerprint in the large and small ribosome subunit of bacterial species profiled, which point to a possible method for the identification of different bacterial species via ribosomal protein mass fingerprinting. In addition, existence of unique ribosomal protein mass fingerprint for the large and small ribosome subunit in each bacterial species profiled also highlighted that while the general structure and function of the ribosome is conserved across species, small differences in structure and function may exist in ribosomes of different bacterial species.

Table 3: Comparison of molecular mass (Da) of ribosomal proteins in the small ribosome subunit of archaeal species

Protein	***Sulfolobus tokodaii***	***Pyrococcus horikoshii***	***Methanococcus jannaschii***	***Halobacterium salinarum* NRC-1**
S1P				
S2P	25523.49	23339.28	25707.9	27207.57
S3P	25108.61	23452.26	23325.27	33070.81
S4P	20661.86	21366.85	22050.31	19323.32
S5P	23899.1	26615.91	23838.86	23007.9
S6P				
S7P	21893.61	24946.03	21742.16	22984.64

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S8P	15022.77	14735.19	14625.3	10877.15
S9P	15723.47	15306.86	15246.06	14537.36
S10P	11997.19	11802.75	12215.24	11466
S11P	14446.69	14744.9	13996	11231.56
S12P	16336.45	16767.92	16828.81	15488.77
S13P	19682.61	16946.86	17380.28	18917.5
S14P	6782.97	6625.98	6192.49	6077.83
S15P	17581.62	18698.03	17845.02	17714.42
S16P				
S17P	13235.55	13703.97	13265.54	11973.4
S18P				
S19P	16452.47	15345.28	17680.16	15917.76
S20P				
S21P				
S3AE	22179.76	22929.11	25776.87	23362.6
S4E	20261.63	28099.96	27658.66	25023.22
S6E	23618.37	13879.33	14371.6	13742.8
S8E	14280.55	14261.66	14539.04	13514.94
S17E	9561.96	8039.49	7641.07	6970.86
S19E	17741.96	17390.22	17029.66	16442.13
S24E	13413.49	11793.66	11841.71	11495.71
S25E	12233.35			
S26E	10834.82			
S27E	7292.76		6797.24	5927.57
S27AE	7539		7048.42	5026.44
S28E	9419.86	8086.27	8750.31	7819.79
S30E	6365.49			

Table 3 shows the calculated molecular mass of ribosomal proteins of the small ribosome subunit of archaeal species *Sulfolobus tokodaii*, *Pyrococcus horikoshii*, *Methanococcus jannaschii*, and *Halobacterium salinarum* NRC-1. Specifically, the data indicated that unique molecular mass existed for individual ribosomal protein of the small ribosome subunit of archaeal species that differed from that of the same protein in another species. Thus, similar to the case in bacterial species, unique ribosomal protein mass fingerprints also existed for the small ribosome subunit of archaeal species which could find use in microbial identification.

Table 4: Comparison of molecular mass (Da) of ribosomal proteins in the large ribosome subunit of archaeal species

Protein	***Sulfolobus tokodaii***	***Pyrococcus horikoshii***	***Methanococcus jannaschii***	***Halobacterium salinarum* NRC-1**
L1P	24866.4	24398.62	26505.54	23092.49

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L2P	25400.41	26142.3	26103.46	25449.48
L3P	38584.17	40814.96	38117.42	36568.73
L4P	30151.53	28681.55	27810.44	26658.37
L5P	20013.47	21610.23	22168.66	19506.51
L6P	21123.39	21253.77	20655.11	19607.49
L9P				
L10P	36796.86	37513.52	36750.94	37213.13
L11P	18237.22	17691.82	17490.34	17038.54
L7/L12P	11487.14	11762.23	10362.74	11561.85
L13P	16508.75	16298.21	15785.6	15893.7
L14P	15509.23	15548.24	14270.98	14375.53
L15P	16594.33	17463.35	16106.96	16728.05
L16P	20110.8			19672.44
L17P				
L18P	21902.56	23700.37	22354	19811.02
L19P	17674.68	17997.39		
L20P				
L21P	11815.02	12554.85		
L22P	17852.86	17688.61	18006.56	17145.02
L23P	9416.22	9820.67	9855.53	9380.52
L24P	9910.55	14757.52	14102.7	13369.4
L25P				
L27P				
L28P				
L29P	10277.15	8037.66	8081.64	7883.84
L30P	18410.83	17667.9	17586	16429.17
L31P				
L32P	15463.43	15702.7		
L33P				
L34P	10225.98	11229.64		
L35P				
L36P				
L7AE	13569.81	13553.88	12685.87	12730.03
L13E	9225.89			
L14E	10832.86	8897.6	8739.36	
L15E	25429.56	22631.74	22578.48	22687.55
L18E	13334.89	13611.96	13532.12	12591.03
L18AE	10167.05	9384.99	8841.53	
L19E			17598.07	16730.2
L21E			11329.32	10587.55
L24E	6993.42	8017.4	8248.61	6999.52

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L30E	11615.67	10765.66	12134.23	
L31E	10403.5	11141.3	10187.23	10223.23
L32E			17274.27	26031.28
L34E			10523.63	
L35AE		9750.54		
L36AE	11136.29	12867.09	11029.11	6771.54
L37E	7050.31	7297.68	7257.65	6357.11
L37AE	7891.31	9321.26	10183.21	
L39E	6134.26		6274.79	6097.06
L40E	6430.82	5884.97	5617.86	5435.21
L41E			3088.77	

Table 4 shows the calculated molecular mass of ribosomal proteins of the large ribosome subunit of archaeal species. Unique molecular mass of each ribosomal protein in the large ribosome subunit of individual archaeal species pointed to the existence of unique ribosomal protein mass fingerprint of the large ribosome subunit of archaeal species, which could be used in microbial identification through MALDI-TOF MS profiling of solubilized ribosomal proteins. Existence of unique ribosomal protein mass fingerprint of the large and small ribosome subunit of archaeal species also highlighted that small differences in structure and function of the ribosome subunits likely existed between different archaeal species due to unique sets of ribosomal proteins of different sequence and mass.

Table 5: Comparison of molecular mass (Da) of ribosomal proteins in the small ribosome subunit of eukaryotic species

Protein	*Neurospora crassa*	*Fusarium graminearum*	*Cryptococcus neoformans*	*Yarrowia lipolytica*
SA	31500.52	31683.46	31485.43	29326.8
S2	28756.23	28003.36	27647.3	27872.31
S3	28666.74	28569.68	27862.18	26920.32
S3A	29068.73	29080.7	29388.02	29064.69
S4	29596.47	29589.42	29659.53	29194.97
S5	23680.17	23807.17	22659	23218.79
S6	27330.9	27269.78	26725.12	27431.74
S7	22872.42	23120.49	22446.97	21790.41
S8	23011.13	23089.1	23803.23	21963.03
S9	21806.42	21960.51	22237.83	22113.42
S10	18431.71	18741.2	17008.23	16266.22
S11	18428.59	18525.62	17545.45	17873.91
S12	16229.63	16179.42	15977.28	15259.41

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S13	16872.85	16848.76	16971.84	16861.73
S14	15997.39	16054.38	15735.01	15402.57
S15	17382.31	17494.36	17127.97	16260.06
S15A	14820.34	14815.35	14455.96	14789.18
S16	15723.52	15924.64	15422.1	15594.37
S17	16942.46	17026.53	16304.77	16081.58
S18	17768.52	17870.7	17879.74	17672.62
S19	16669.84	16641.78	18102.07	16247.35
S20	13225.56	12918.99	13395.56	12972.03
S21	9660.95	9658.89	9469.69	9684.76
S23	15909.58	15744.47	16019.69	15896.54
S24	15562.2	15528.1	15377.93	15097.47
S25	10797.67	10485.4	10271.2	11841.04
S26	13545.93	13281.77	14502.12	13613.13
S27	8897.55	8853.54	8969.57	8982.53
S27A	17738.67	17741.73	17601.78	17166.99
S28	7720.94	7766	7842.13	7720.96
S29	6500.44	6692.75	6601.68	6623.61
S30	6910.19	6861.15	7296.59	6860.12

Table 5 shows the calculated molecular mass of ribosomal proteins of the small ribosome subunit of eukaryotic species: *Neurospora crassa, Fusarium graminearum, Cryptococcus neoformans,* and *Yarrowia lipolytica*. Distinct molecular mass of individual ribosomal proteins of the small ribosome subunit of profiled eukaryotic species highlighted that unique ribosomal protein mass fingerprint existed for individual species. Given the large number of ribosomal proteins in the small ribosome subunit of eukaryotes, ribosomal protein mass fingerprint of the small ribosome subunit could find use in microbial identification especially in the case of fungus and molds that lack distinguishing phenotypic characteristics. Existence of unique ribosomal protein mass fingerprint of small ribosome subunit of eukaryotes also pointed to possible structural and functional differences in the small ribosome subunit of different eukaryotic species that did not affect basic processes of protein translation.

Table 6: Comparison of molecular mass (Da) of ribosomal proteins in the large ribosome subunit of eukaryotic species

Protein	*Neurospora crassa*	*Fusarium graminearum*	*Cryptococcus neoformans*	*Yarrowia lipolytica*
L3	44035.18	44080.19	43788.44	43957.7
L4	38813.91	37671.4	39499.57	39584.76
L5	34411.69	34758.19	34659.36	33855.25
L6	22475.12	22245.72	25435.62	20471
L7	28687.61	28486.25	28268.14	28319.03

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L7A	29366.99	29350.86	29442.54	29020.1
L8	27356.43	27561.71	27457.49	27412.59
L9	21752.18	21871.21	20943.43	21337.63
L10	25325.46	25348.34	24845.99	25291.32
L10A	24126.39	24131.32	25433.96	24307.68
L11	20084.16	19939.07	19781.92	19668.71
L12	17693.54	17690.49	17561.34	17618.41
L13	23876.73	23858.54	23330.77	23442.08
L13A	22900.92	22814.8	21940.87	22500.47
L14	15853.62	16663.28	15458.06	15657.26
L15	24190.04	24017.93	23644.37	24072.76
L17	20763.77	20690.71	20499.64	20550.38
L18	20602.26	20619.06	21350.01	20832.38
L18A	20342.93	20534.77	21253.93	20414.72
L19	22348.05	21854.27	22704.5	21698.29
L21	18214.3	18077.99	18551.57	18574.63
L22	14209.47	14237.21	14167.3	13112.91
L23	14699.38	14654.38	14664.32	14282.86
L23A	17119.07	16737.64	17252.22	15937.65
L24	17611.48	18039.86	16802.66	17330.15
L26	15330.84	15388.77	14985.38	14145.39
L27	15722.43	15705.47	15769.6	15730.49
L27A	16597.27	16905.73	15973.63	16460.05
L28	15979.42	17113.29	16632	
L29	7464.51	7487.61	6910.9	7176.3
L30	11702.69	11842.83	12343.27	11474.34
L31	14065.36	14007.25	14370.66	13451.67
L32	14964.7	15032.7	14707.3	14764.36
L34	13194.51	13287.57	12597.05	12325.56
L35	14409.12	14364.94	14679.32	14172.68
L35A	12178.12	12201.15	11884.82	12196.19
L36	11555.6	11777.85	12078.27	11025.89
L36A	11912.15	12112.43	12146.36	12302.6
L37	10232.88	10730.35	10193.87	9515
L37A	10132.89	10161	10076.78	10178.83
L38	9148.88	8936.54	10687.67	7998.37
L39	6265.45	6239.37	6361.57	6196.33
L40	14637.2	14596.15	14653.14	14538.05
L41	3367.24	3239.06		3335.18
LP0	33534.13	33379.07	33424.43	33889.5
LP1	11043.28	11007.12	10830.09	10442.57

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LP2	11102.31	11036.08	11074.25	10859.9
LP3				

Table 6 shows calculated molecular mass of ribosomal proteins of the large ribosome subunit of eukaryotic species. Similar to the case of the small ribosome subunit of eukaryotes, unique molecular mass also existed for individual ribosomal protein of the large ribosome subunit of different eukaryotic species. Thus, unique ribosomal protein mass fingerprint of the large and small ribosome subunit existed for individual eukaryotic species, which offers an alternative approach for the identification of hard to discriminate eukaryotic species such as fungus and molds. In addition, existence of unique ribosomal protein mass fingerprint of the large ribosome subunit of eukaryotic species also pointed to possible differences in structure and function of the large ribosome subunit. However, these differences in structure and function should be small and do not affect the main function of the ribosome: translation. Moreover, the general structure of eukaryotic ribosome should be similar while allowing small differences in less essential areas to exist between different eukaryotic species.

Overall, distinct molecular mass of ribosomal proteins in the large and small ribosome subunit of bacterial, archaeal and eukaryotic species highlighted the existence of unique ribosomal protein mass fingerprint of species in the three domains of life. Such distinctive ribosomal protein mass fingerprint offered possibilities in the identification of different bacterial, archaeal and eukaryotic species through gel-free mass spectrometric profiling of solubilized ribosomal proteins via MALDI-TOF MS. Specifically, microbes could be identified by comparing the profiled ribosomal protein mass spectrum with those of known microorganisms catalogued in a reference database. Besides possibilities in microbial identification, existence of unique ribosomal protein mass fingerprint of the large and small ribosome subunit also pointed to differences in the structure and function of the large and small ribosome subunit in different species. Specifically, while the general structure and function of the ribosome subunit should be similar given high level of conservation, small structural and functional differences in the ribosomes could nevertheless exist between different species.

Conclusions

Distinct molecular mass existed for individual ribosomal protein of the large and small ribosome subunit of different species from the three domains of life. Thus, unique ribosomal protein mass fingerprints existed for the large and small ribosome subunit that provided the conceptual and biological basis for a new approach towards microbial identification. Specifically, the approach is suited for gel-free mass spectrometric profiling of all solubilized ribosomal proteins of the large and small ribosome subunit that helps generate an experimental ribosomal protein mass fingerprint that could be compared with those of known microorganisms catalogued in a reference database. Similarly, comparison of experimental ribosomal protein mass fingerprint with theoretical ones of known microorganisms catalogued in a database could also be used for

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identification. Use of the gentle ionization method of matrix-assisted laser desorption/ionization (MALDI) coupled with a time-of-flight (TOF) mass analyser could help provide an approach towards the profiling of solubilized ribosomal proteins. Beyond possible use in microbial identification, existence of unique ribosomal protein mass fingerprint for individual species also highlighted possible existence of small structural and functional differences in the small and large ribosome subunit of different species. Specifically, the general structure and function of the ribosome is highly conserved and thus similar across species in different domains of life, but small differences in structure and function from ribosomal proteins of differentiated sequences could arise that did not impact on the overall purpose of the ribosome: protein translation. Thus, ribosomes of different species might be differentiated in structure and function to a small extent while maintaining the same function.

Supplementary materials

Comparison of molecular mass of ribosomal proteins from different species is appended to this manuscript as an Excel file.

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Conflicts of interest

The author declares no conflicts of interest.

Author's contribution

The author conceived the idea, collected and analysed the data, and wrote the manuscript.

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