A new approach towards prime numbers

All the natural numbers > 1, comprising prime and composite numbers, can be represented by even and odd integers $2n$ and $2n + 1$ respectively, where $n = 1, 2, 3, \ldots$. As regards even integers $2n$, we know that for $n = 1$, there is a prime 2 whereas for $n = 2, 3, 4 \ldots$ there are composite numbers 4, 6, 8, ... However, we do not know any method to find the values of n for which odd integers $2n + 1$ will represent prime and composite numbers.

To answer the question raised above, we consider the set S of odd integers > 1 and form the subset of odd composite numbers $S.S = S^2$ as given below. Thus, if

$$S = \{2n + 1; n = 1, 2, 3, \ldots\}, \quad (1)$$

then

$$S^2 = \{(2j + 1)\cdot(2k + 1); j = 1, 2, 3, \ldots, k \geq j\}, \quad (2)$$

where $(2j + 1)\cdot(2k + 1)$ is taken to denote usual multiplication of odd integers > 1.

However, for getting the required expression for n, we simplify (2) and write it as

$$S^2 = \{2\,(2jk + j + k) + 1; j = 1, 2, 3, \ldots, k \geq j\}$$

or,

$$S^2 = \{2n + 1; n = 2jk + j + k, j = 1, 2, 3, \ldots, k \geq j\}. \quad (3)$$

Thus, when n is of the form

$$n = 2jk + j + k\,. \quad (4)$$

$2n + 1$ will represent odd composite numbers for $j = 1, 2, 3, \ldots, k \geq j$. Obviously, for the remaining values of n, i.e. when n is not of the form (4), $2n + 1$ will represent odd primes. It may be noted here that on putting $j = k = 1$ in (4) we get $n = 4$ and so on putting $n = 4$ in $2n + 1$, we get the smallest odd composite number 9. But, since 4 is the minimum value of n obtainable from (4), $n = 1, 2, 3$ cannot be derived from (4). Hence on putting $n = 1, 2, 3$ in $2n + 1$, we will get the first three odd primes 3, 5, 7.

However, for generating all the odd primes ≤ any odd integer N, we, from (4), will find out all the values of n in the range $1 \leq n \leq (N - 1)/2$ by assigning all the possible permutations and combinations of the values of $j = 1, 2, 3 \ldots, k \geq j$. These values of n, derived from (4), when substituted in $2n + 1$, will yield odd composite numbers ≤ N. Obviously, the remaining values of n, not obtainable from (4), in the range $1 \leq n \leq (N - 1)/2$, when substituted in $2n + 1$, will yield odd primes ≤ N.

Remark: It may be mentioned here that we cannot derive any expression of the form (4) for which n will directly generate odd primes through $2n + 1$. The reason for this is that it is the composite numbers (and not the primes) which, besides the usual operation of successive addition of 1, can also be generated by the operation of multiplication.

New definition: In the light of above remark, we can have the following definition of prime and composite numbers.

An integer $n > 1$, which, besides the usual operation of successive addition of 1, can also be generated by the operation of multiplication of at least two integers (other than 1 and n), is a composite number, and an integer $n > 1$, which is not composite, is a prime.

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Protein profile analysis of *Listeria monocytogenes* strains from the tropics

Though *Listeria monocytogenes* has been considered as a serious food-borne pathogen in developed countries, the incidence of this organism in tropical foods has been reported to be very low[1,2]. Being an opportunistic pathogen, *L. monocytogenes* causes severe illness, mainly in pregnant women and their foetuses, the elderly and immunocompromised patients. The disease is primarily transmitted through contaminated food[3]. Though *Listeria* spp. is isolated from a variety of raw and processed food, the source of contamination is difficult to trace. Typing methods may be helpful to identify *Listeria* clones and their environmental niches that are the greatest threat to man[4]. Various typing methods like serotyping[5], phage typing[6] and random amplification of polymorphic DNA (RAPD)[7] are being used for typing of *L. monocytogenes* and to trace the source of contamination.

Protein expression is a phenotypic character, which is regulated by both genotype and environmental factors. In order to compare the homogeneity and heterogeneity among the different isolates of *L. monocytogenes*, the cell-associated protein profiles of *L. monocytogenes* isolated from clinical, veterinary and food samples in India were studied. Aeration has an effect on haemolytic activity, growth and catalase production[8]. Cellobiose is known to repress the expression of virulence factor in *L. monocytogenes*[9]. Hence, the effect of aeration and the presence of cellobiose in the medium on protein profile were also investigated.

During the period from March 1997 to June 2000, 633 clinical samples and 320 food samples were processed and four strains *of L. monocytogenes* were isolated. Among these four strains, two were from human clinical samples (from placenta) and two were from food samples

(one each from clams and flat fish). Four isolates from veterinary samples (infected sheep) were also included in the study (cultures provided by Haryana Agricultural University, Hisar, India). These strains along with a standard strain of *L. monocytogenes* (NCTC 7973) and an atypical isolate (from placenta), that showed all biochemical characteristics of *L. monocytogenes*, except α-methyl D-mannoside fermentation, were used in this study.

The cell-associated protein preparation protocol of *L. monocytogenes* was adapted from Sokolovich *et al.*[10], Chanphong and Sirirat[11] and Bohne *et al.*[12]. Standard strain of *L. monocytogenes* and isolates from food, clinical and veterinary samples were grown in 2 ml of Brain Heart Infusion (BHI) broth at 37°C for 6–8 h. Next, 200 µl of BHI culture was inoculated into 20 ml of BHI broth, incubated for 10–12 h at 37°C. The cultures of the isolates were also grown with and without aeration in BHI broth at 37°C for 10–12 h. To know the effect of cellobiose on the expression of cell-associated proteins, cultures were also grown in BHI broth containing 1 and 10 mM cellobiose. The cultures were centrifuged at 8000 rpm for 20 min at 4°C. The cell pellet was washed with cold 20 mM *Tris* buffer (*Tris* base 4.844 g/l, distilled water 1 l, pH 7.2) and the washing process was repeated twice. Finally, the pellet was suspended in sample buffer (10% SDS 4 ml, glycerol 2 ml, 1 M *Tris*-HCl 1.2 ml–pH 6.8, distilled water 2.8 ml) and 10 µl β-mercaptoethanol, heated in the dry broth for 10 min at 95°C and stored at – 20°C till further use.

Protein profiles of cell lysate were generated by analytical sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) as described by Harlow and Lane[13], using 12% gel. Samples (20 µl) and low molecular-weight markers (15 µl, Pharmacia LKB Biotechnology Inc., USA) were loaded in the gel. Electrophoresis was initialized at 80 V, till the dye front reached separating gel, after which the voltage was increased to 120 V. After electrophoresis, the gel was stained by Coomassie blue stain, destained and photographed.

Two isolates of *L. monocytogenes* from clinical samples (placenta) and one of the isolates of *L. monocytogenes* from food samples (clams) showed similar bands in major proteins (Figure 1). The isolate of *L. monocytogenes* from food sample (flat fish) showed a prominent band of molecular weight ≅ 30.1 kDa (Figure 1). Heterogeneity was observed among the two *L. monocytogenes* strains from fish samples. The atypical strain showed totally different protein profile. This organism was subsequently confirmed as not belonging to *Listeria* spp. by Pathalert Listeria ELISA kit (marketed by Merck Diagnostics, Germany) and also PCR-targetting of a fragment of *iap* gene[14]. All the four veterinary isolates (sheep) showed similar bands (Figure 2). SDS–PAGE has been used to fingerprint bacterial proteins. Tabouret *et al.*[15] analysed the surface proteins of *Listeria* in relation to species, serovar and pathogenicity by using SDS–PAGE. Their results showed a high degree of homogeneity among all strains of the same serovar and heterogeneity between serovar 1/2 and 4 b. In this study, the two clinical isolates and the isolate from clams belonged to serotype 1, while the isolate from flat fish belonged to serotype 4. Thus the protein profile difference observed in this study may be related to the serotype difference.

To study whether the 30.1 kDa protein was extracellular, SDS–PAGE of cell-supernatant protein and cell pellet-associated protein was done. The results showed that, the protein was indeed cell-associated. Benedict and Schultz[8] and Benedict[16] showed that aeration increased the growth of *L. monocytogenes* and also the production of catalase, but the reduced aeration increased haemolytic activity. In the present study aeration had no significant effect on the expression of 30.1 kDa protein (Figure

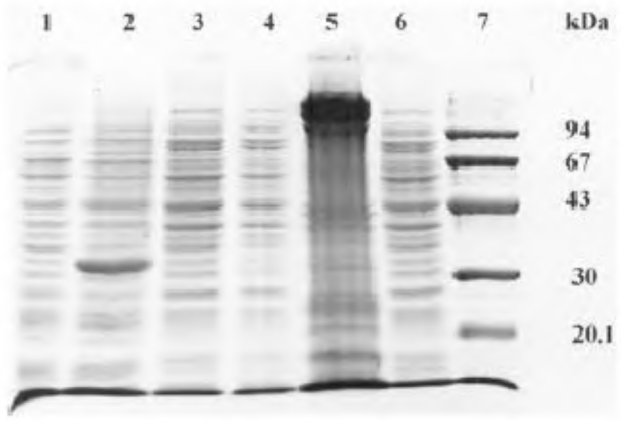

Figure 1. SDS–PAGE for cell-associated proteins of *Listeria monocytogenes*. Isolates were from clams (lane 1), flat fish (lane 2) and placenta (lanes 3 and 4). Lane 5, Atypical isolate; lane 6, *L. monocytogenes* (NCTC 7973); lane 7, Molecular weight marker.

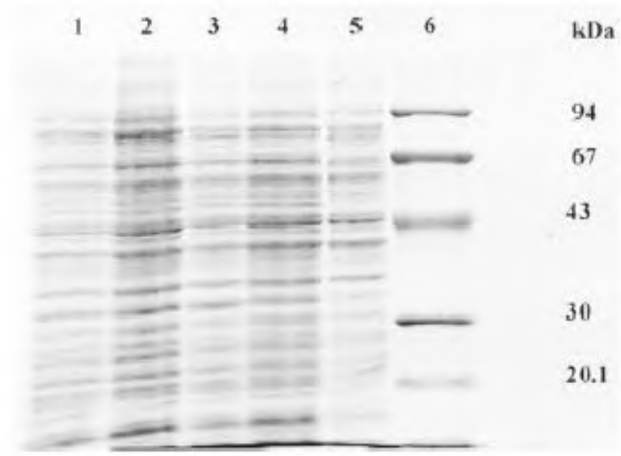

Figure 2. SDS–PAGE for cell-associated proteins of *L. monocytogenes*. Isolates were from veterinary samples (lanes 1–4). Lane 5, *L. monocytogenes* (NCTC 7973); lane 6, Molecular weight marker.

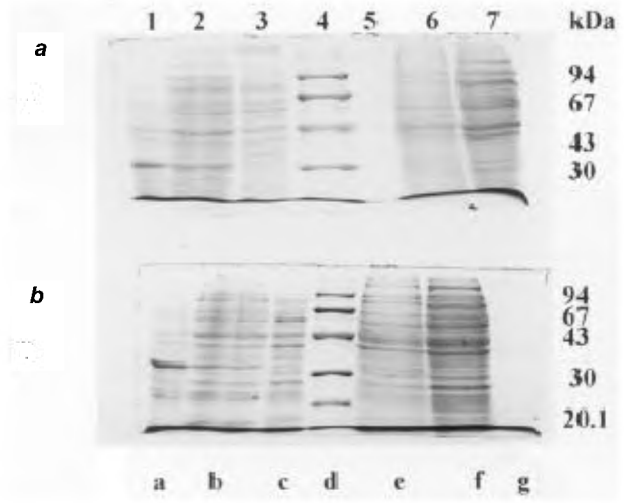

Figure 3. SDS–PAGE for cell-associated and cell-supernatant proteins of *L. monocytogenes* isolate from flat fish using (***a***) 10% and (***b***) 12% gel. Lanes 1 and a, Isolate from flat fish grown without aeration (cell surface); lanes 2 and b, Isolate from flat fish grown with aeration (cell surface); lanes 3 and c, *L. monocytogenes* (NCTC 7973) without aeration (cell surface); lanes 4 and d, Molecular weight marker; lane 5, Blank; lanes 6 and e, Isolate from flat fish (cell supernatant); lanes 7 and f, *L. monocytogenes* (NCTC 7973; cell supernatant); lane g, BHI ppt.

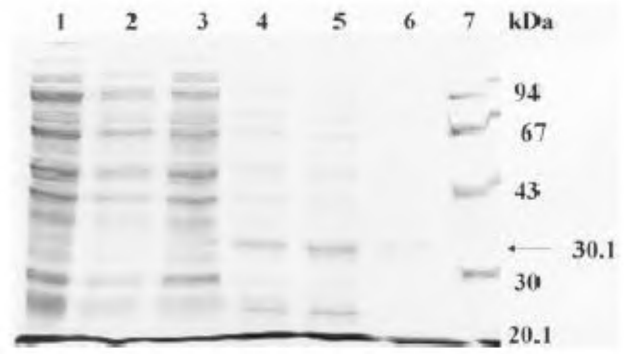

Figure 4. SDS–PAGE for cell-associated proteins of *L. monocytogenes* isolate from flat fish grown in the presence and absence of cellobiose. Lane 1, *L. monocytogenes* (NCTC 7973; BHI); lane 2, *L. monocytogenes* (NCTC 7973; 1 mM cellobiose); lane 3, *L. monocytogenes* (NCTC 7973; 10 mM cellobiose); lane 4, Isolate from flat fish (BHI); lane 5, Isolate from flat fish (1 mM cellobiose); lane 6, Isolate from flat fish (10 mM cellobiose); lane 7, Molecular weight marker.

3). Expression of major virulence determinants in *L. monocytogenes* was repressed by the plant-derived molecule, cellobiose[9]. In order to know the effect of cellobiose on the expression of 30.1 kDa proteins, profiles were generated with cellobiose in the medium. It was observed that there was no appreciable difference in the expression of 30.1 kDa protein in the presence and absence of cellobiose (Figure 4). However, 10 mM cellolobiose appeared to suppress the expression of many proteins in the flat-fish isolate.

Serotyping of *L. monocytogenes* is done only in a few laboratories and therefore, protein-profile analysis may be a useful tool to study heterogeneity among clinical and environmental isolates of *L. monocytogenes*. Protein-profile analysis will also be useful to identify any unusual protein-associated *L. monocytogenes* strain.

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Heavy-metal phytotoxicity induces oxidative stress in a moss, *Taxithellium* sp.

Highly reactive free radicals are produced with the exposure of plants to a range of abiotic stresses which include water, salt, heavy-metal toxicity, etc. These reactive oxygen species have been implicated directly with molecular damage in plant cells[1]. Heavy metals are integrated components of the ecosystem. Though some heavy metals are essential as micronutrients, uptake of higher concentrations of heavy metal is found to be toxic for plants. Certain metals are known to produce/act as catalysts for the production of free radicals in biological systems[2,3]. Many heavy metals like Fe, Cu, Cd, Cr, Zn, etc. have been shown to cause oxidative damage in various higher plants[4–8]. Bryophytes are important members of the lower plant community and are also found to be sensitive to various abiotic stresses[9–11]. However, little is known about the mechanism of oxidative stress in bryophytes under heavy-metal exposure. The present investigation was carried out to study the induction of oxidative stress in *Taxithellium* sp., a bryophyte subjected to heavy-metal treatment.

Taxithellium sp. was collected during September 2001 from the Botanical Garden of Assam University, Silchar, and brought to the laboratory in polythene bags. After thorough washing in running tap water and distilled water, moss was transferred to petri plates containing various concentrations (0, 1, 10, 100 and 1000 µM) of potassium dichromate ($K_2Cr_2O_7$), copper sulphate ($CuSO_4$) and zinc chloride ($ZnCl_2$). Petri plates were transferred to a growth chamber provided with cool, fluorescent, white tube lights (Philips TLD, 20 W) with a photon flux density of 52 $\mu Em^{-2}s^{-1}$ (PAR) and kept at 22 ± 2°C. After 24 h of treatment, plant material was taken for biochemical and enzymic estimations.

Pigment content was estimated according to the method of Arnon[12] and Lichtenthaler *et al.*[13]. Measurement of proline, total peroxide and thiobarbituric acid reactive substance (TBARS) was done according to the method of Bates *et al.*[14], Sagisaka[15] and Heath and Packer[16] respectively. Extraction and assay of catalase (CAT), guaiacol peroxidase, (GPX) and superoxide dismutase (SOD) were done according to the method of Chance and Maehly[17] and Giannopolitis and Ries[18] respectively. Data presented are means of four separate experiments ± standard errors.

Figure 1 shows the effect of heavy metals on the pigment status of moss. With increase in concentration of all the

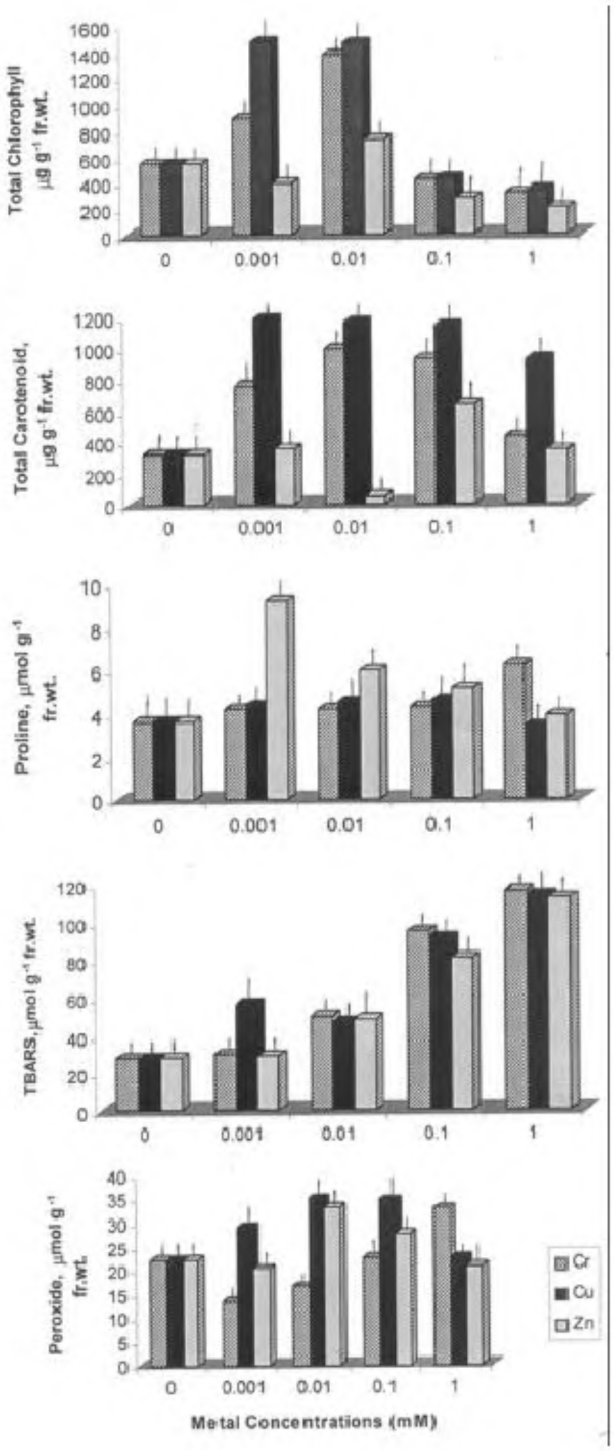

Figure 1. Change in chlorophyll, carotenoid, proline, peroxide and TBARS content in *Taxithellium* sp. under heavy-metal treatment. Data presented are mean ± standard errors.